



## Long- term manure exposure increases soil bacterial community potential for plasmid uptake

Musovic, Sanin; Klümper, Uli; Dechesne, Arnaud; Magid, Jakob; Smets, Barth F.

*Published in:*  
Environmental Microbiology Reports

*Link to article, DOI:*  
[10.1111/1758-2229.12138](https://doi.org/10.1111/1758-2229.12138)

*Publication date:*  
2014

[Link back to DTU Orbit](#)

*Citation (APA):*  
Musovic, S., Klümper, U., Dechesne, A., Magid, J., & Smets, B. F. (2014). Long- term manure exposure increases soil bacterial community potential for plasmid uptake. *Environmental Microbiology Reports*, 6(2), 125-130. DOI: 10.1111/1758-2229.12138

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 Long-term manure exposure increases soil bacterial community potential for plasmid  
2 uptake

3 **Sanin Musovic<sup>1</sup>, Uli Klümper<sup>1</sup>, Arnaud Dechesne<sup>1</sup>, Jakob Magid<sup>2</sup> and Barth F. Smets<sup>1</sup>**

4 Department of Environmental Engineering, Technical University of Denmark, Miljøvej, DK-  
5 2800 Kgs. Lyngby, Denmark,<sup>1</sup> and Department of Agriculture and Ecology, Plant and Soil  
6 Science, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-  
7 1871 Frederiksberg C, Denmark<sup>2</sup>

8

9 Correspondence

10 Barth F. Smets, Technical University of Denmark, Department of Environmental  
11 Engineering, Bygningstorvet Building 115, 2800 Kgs. Lyngby, +45 45252230,  
12 [bfsm@env.dtu.dk](mailto:bfsm@env.dtu.dk)

13

14 Running title: Manure increases community plasmid uptake

15 **Abstract:**

16 Microbial communities derived from soils subject to different agronomic treatments were  
17 challenged with three broad host range plasmids, RP4, pIPO2tet, and pRO101, via solid  
18 surface filter matings to assess their permissiveness. Approximately 1 in 10,000 soil  
19 bacterial cells could receive and maintain the plasmids. The community permissiveness  
20 increased up to 100% in communities derived from manured soil. While the plasmid  
21 transfer frequency was significantly influenced by both the type of plasmid and the  
22 agronomic treatment, the diversity of the transconjugal pools was purely plasmid  
23 dependent and was dominated by  $\beta$ - and  $\gamma$ -Proteobacteria.

24

25 **Text**

26 Rapid adaption of bacterial communities to changing environmental conditions is believed  
27 to rely on lateral transfer of mobile genetic elements, such as plasmids, as one  
28 indispensable mechanism (Sørensen et al., 2005; Thomas and Nielsen, 2005; Heuer and  
29 Smalla, 2012). One of the crucial parameters that determines the extent of conjugal  
30 plasmid transfer is community permissiveness, defined as the fraction of a microbial  
31 community able to receive a given plasmid (Musovic et al., 2010). In agricultural soils,  
32 seasonal application of manure and fertilizers provokes intense and immediate changes to  
33 soil physical-chemical conditions that might modulate horizontal gene transfer (HGT).  
34 Indeed, both an increased nutrient availability (van Elsas et al., 2003) and the introduction  
35 of selective stressors like metals or antibiotics (Newby and Pepper, 2002; Heuer et al.,  
36 2011) may lead to increased rates of plasmid transfer. Apart from this immediate  
37 stimulation of HGT, it remains unknown whether agronomic soil treatment may have long-  
38 term effects on permissiveness. To test this, we investigated permissiveness towards  
39 three broad-host-range plasmids (Table 1) in four communities isolated from agricultural  
40 soils subjected to different long-term seasonal treatments (Table 2).

41 Microbial communities from three plots (Untreated, Manured and Nitrate-Phosphate-  
42 Potassium-fertilized (NPK)), at the long-term CRUCIAL experimental site (Taastrup,  
43 Denmark) (Magid et al., 2006; Poulsen et al., 2013), were sampled (SI2) in order to assess  
44 permissiveness. Treatments at the field site were in place for 8 years at the time of  
45 sampling. The manure derived from a conventional dairy cow farm. The estimated  
46 accumulative applications of C-N-P-K were estimated at 62800-3768-1184-2979  
47 (manured), 0-840-120-400 (NPK) and 0-0-0-0 (untreated) kg/ha respectively (Magid et al.,  
48 2006).

49 As reference, soil from an untreated plot of the well-known Rothamsted Park Grassland  
50 (Rothamsted, United Kingdom) (Silvertown et al., 2006) site was included. From the  
51 chosen soils, indigenous bacterial communities were isolated by Nycodenz®-extraction  
52 (Musovic et al., 2010) and challenged with exogenous plasmids via solid-surface filter  
53 matings (Musovic et al., 2010). *Pseudomonas putida* KT2442 (Bagdasarian et al., 1981)  
54 served as the plasmid donor strain (Table 1). Conjugation events were detected by  
55 epifluorescence microscopy, allowing their quantification (Musovic et al., 2010).

56 For all tested combinations, community permissiveness ranged from  $8.24 \cdot 10^{-5}$  to  $4.56 \cdot 10^{-4}$   
57 conjugations per recipient (Figure 1). Permissiveness was consistently higher (up to 3  
58 times) for the soil-derived pIPO2tet plasmid than for the other two IncP plasmids, RP4 and  
59 pRO101. Therefore, for both sampling sites, the introduced plasmid significantly influenced  
60 the permissiveness ( $p_{\text{CRUCIAL}} < 0.001$ ;  $p_{\text{Rothamstead}} < 0.001$ ). Additionally, in CRUCIAL soils, a  
61 significantly higher permissiveness was measured for the manure treated community  
62 compared to the untreated control ( $p_{\text{RP4}} = 0.041$ ;  $p_{\text{pIPO2tet}} = 0.001$ ). Previous studies have  
63 suggested that manure treatment may result in hot-spots of gene transfer due to increased  
64 nutrient availability and cell density (van Elsas et al., 2003) with transfer frequencies  
65 increasing by up to one order of magnitude (Götz and Smalla, 1997). However, this does  
66 not explain why we observed stimulation of permissiveness by manuring because, in our  
67 assay, heterogeneities in nutrient or cell density mating were excluded as matings were  
68 carried out under standard nutritional conditions on soil extract medium (Musovic et al.,  
69 2010). While raised levels of plasmid shuttled tetracycline resistance genes were shown to  
70 occur in soils treated with piggery manure slurry (Agersø et al., 2006) or chicken waste  
71 (You et al., 2012) in selective and enriching environments, this study is the first to indicate

72 that long-term manure treatment also changes the community permissiveness towards  
73 newly introduced plasmids under neutral conditions.

74 Nutrient addition has previously been shown to enhance gene transfer frequency in soils  
75 (Smets et al., 1995; Nielsen and van Elsas, 2001). To study the effect of fertilization, we  
76 investigated the permissiveness of RP4 in the NPK-fertilized soil bacterial community.  
77 Permissiveness in the NPK-treated soil was similar to the untreated control ( $p=0.79$ ) and  
78 significantly lower than in the manured one ( $p=0.016$ ). Therefore, higher activity through  
79 previous nutrient addition is not the reason for increased permissiveness in manured soil.  
80 It thus seems that increased permissiveness was not due to immediate nutrient effects, or  
81 increased cell density in hot-spots. However, increased permissiveness might have been  
82 intrinsic to the community. Hence, we examined the diversity of the transconjugal pools, to  
83 test whether they differ between soil treatments.

84 Random transconjugants were isolated from the matings using micromanipulation  
85 (Musovic et al., 2010) to analyze, if apart from increased transfer frequencies, the  
86 phylogenetic composition of the transconjugal pool changed due to long-term manure  
87 application. Successfully isolated transconjugants were subjected to 16S rRNA  
88 sequencing (Musovic et al., 2010). Sequences were analyzed using mothur v.1.30.0  
89 (Schloss et al., 2009) and the SILVA database (Quast et al., 2013). The sequences have  
90 been submitted to GenBank and can be accessed under number KF590708 - KF591079.

91 Principal Coordinate Analysis (PCoA) revealed transconjugal pools separating primarily by  
92 introduced plasmid (Axis 1, Figure 2). Independent of sampling site or treatment, pRO101,  
93 RP4 and pIPO2tet were associated with significantly different transconjugal pools as  
94 revealed by AMOVA (Excoffier et al., 1992) ( $p=0.007$ ). Surprisingly, the number of OTUs

95 was lowest for pIPO2tet (SI Table 1), the plasmid exhibiting the highest permissiveness.  
96 Although the effective phylogenetic host range in the tested soils appears smallest for  
97 pIPO2tet, it might exhibit higher transfer frequencies, resulting in a higher total  
98 permissiveness, possibly due to its nature as cryptic plasmid (van Elsas et al., 1998). High  
99 transfer frequencies are especially important for the maintenance of those plasmids not  
100 conferring any beneficial traits to their hosts.

101 The second dimension of PCoA separated all transconjugal pools from the CRUCIAL site  
102 from those from Rothamsted ( $p_{RP4} < 0.001$ ;  $p_{pIPO2tet} < 0.001$ ). This separation likely derives  
103 from differences in their original bacterial community composition. Meanwhile, an earlier  
104 study – comparing deeply sequenced 16S rRNA community libraries – revealed no major  
105 difference in the total bacterial community composition for different treatments at the  
106 CRUCIAL site (Poulsen et al., 2013), hinting towards the observed grouping of those  
107 transconjugal pools.

108 While plasmid and sampling site both affected transconjugal pools, no effect of agricultural  
109 treatment was detected ( $p > 0.9$ ) for any of the plasmids in the CRUCIAL soil. A closer look  
110 at the six corresponding phylogenetic profiles (Figure 3) confirms that transconjugal pools  
111 of the same plasmid within different soil communities are closely related. For instance,  
112 *Enterobacteriaceae* sequences can only be found in both transconjugal pools associated  
113 with pRO101, although their relative fractions differ. All six pools were dominated by  $\beta$ - and  
114  $\gamma$ -Proteobacteria.  $\alpha$ -Proteobacteria, Flavobacteria and Sphingobacteria were detected in  
115 lower abundance, revealing a wide variety of transconjugants (SI Table 1), in spite of the  
116 low number of isolates. High-throughput analysis of transconjugal pools could in the future  
117 lead to new insights in the extent of plasmid transfer in soils.

118 The similarity of transconjugal pools across soil treatments parallels that of the total  
119 bacterial community composition of CRUCIAL soils (Poulsen et al., 2013). Therefore, the  
120 increased community permissiveness in manured soil cannot be explained by difference in  
121 community diversity. Increased seasonal nutrient availability can also be ruled out, since  
122 high permissiveness was not observed for NPK-fertilized soil. Potentially, a higher  
123 indigenous plasmid content in the community associated with manure applications (Marti  
124 et al., 2013), can result in a higher permissiveness towards additional plasmids, by  
125 increasing the mating potential of the plasmid bearing cells. Additionally, the introduction of  
126 diverse plasmids through manure application may increase the community's  
127 permissiveness. Indeed previous hosting of a plasmid has been shown to significantly  
128 increase the permissiveness towards its renewed uptake in a *Dickeya* strain (Heuer et al.,  
129 2010).

130 On the other hand, the periodic introduction of stressors, such as metal ions (Nicholson et  
131 al., 1999) or antibiotics (Christian et al., 2003) present in manure, might lead to selection  
132 mechanisms favoring more robust populations adapted to environmental changes through  
133 increased permissiveness towards foreign DNA uptake (Heuer et al., 2008). These  
134 selection mechanisms for more permissive strains do not have to be associated with  
135 changes in the phylogenetic profile of the community, since permissiveness towards broad  
136 host range plasmids of isolates that are genetically indistinguishable by 16S rRNA analysis  
137 from identical field plot can differ by more than 2 orders of magnitude (Heuer et al., 2010).  
138 Still, the exact mechanisms of the observed enhanced permissiveness are yet to be  
139 elucidated. High-throughput analysis of the transconjugal pools could thus lead to insights  
140 into community dynamics of plasmid transfer in soil.

141 Here, we confirmed that soil communities can serve as significant reservoirs for  
142 exogenous conjugal plasmids. Approximately 1 in every 10,000 indigenous cells could  
143 receive and maintain the studied plasmids. This frequency is increased up to 100% in soils  
144 subject to manure treatment. Such an increase in the permissiveness of soil communities  
145 and therefore in their potential for contributing to the spread of antibiotic resistance genes  
146 is remarkable, making it crucial to elucidate the underlying mechanisms in further research  
147 with higher sampling depth. Additional work investigating dissemination and mobilization  
148 capacity of more diverse genetic elements among bacterial communities from differently  
149 treated soils is therefore necessary.

150

151

152 **Acknowledgements**

153 This work was funded by the Villum Kann Rasmussen Foundation Center of Excellence  
154 CREAM I & II (Center for Environmental and Agricultural Microbiology). We thank Ian M.  
155 Clark for providing the soil samples from Rothamsted, UK, and Lene K. Jensen and Naleli  
156 K. Vad for their technical support.

157 **References**

- 158 Agersø, Y., Wulff, G., Vaclavik, E., Halling-Sørensen, B., and Jensen, L.B. (2006) Effect of  
159 tetracycline residues in pig manure slurry on tetracycline-resistant bacteria and  
160 resistance gene tet(M) in soil microcosms. *Environ. int.* **32**: 876–82.
- 161 Bagdasarian, M., Lurz, R., Rückert, B., Franklin, F.C.H., Bagdasarian, M.M., Frey, J., and  
162 Timmis, K.N. (1981) Specific-purpose plasmid cloning vectors II. Broad host range,  
163 high copy number, RSF 1010-derived vectors, and a host-vector system for gene  
164 cloning in *Pseudomonas*. *Gene* **16**: 237–247.
- 165 Christian, T., Schneider, R.J., Färber, H.A., Skutlarek, D., Meyer, M.T., and Goldbach,  
166 H.E. (2003) Determination of antibiotic residues in manure, soil, and surface waters.  
167 *Acta Hydrochim. Hydrobiol.* **31**: 36–44.
- 168 Van Elsas, J.D., McSpadden Gardener, B.B., Wolters, A.C., and Smit, E. (1998) Isolation,  
169 characterization, and transfer of cryptic gene-mobilizing plasmids in the wheat  
170 rhizosphere. *Appl. Environ. Microbiol.* **64**: 880–889.
- 171 Van Elsas, J.D., Turner, S., and Bailey, M.J. (2003) Horizontal gene transfer in the  
172 phytosphere. *New Phytol.* **157**: 525–537.
- 173 Excoffier, L., Smouse, P.E., and Quattro, J.M. (1992) Analysis of molecular variance  
174 inferred from metric distances among DNA haplotypes: application to human  
175 mitochondrial DNA restriction data. *Genetics* **131**: 479–491.
- 176 Götz, A. and Smalla, K. (1997) Manure enhances plasmid mobilization and survival of  
177 *Pseudomonas putida* introduced into field soil. *Appl. Environ. Microbiol.* **63**: 1980–  
178 1986.
- 179 Heuer, H., Abdo, Z., and Smalla, K. (2008) Patchy distribution of flexible genetic elements  
180 in bacterial populations mediates robustness to environmental uncertainty. *FEMS*  
181 *Microbiol. Ecol.* **65**: 361–71.
- 182 Heuer, H., Ebers, J., Weinert, N., and Smalla, K. (2010) Variation in permissiveness for  
183 broad-host-range plasmids among genetically indistinguishable isolates of *Dickeya* sp.  
184 from a small field plot. *FEMS Microbiol. Ecol.* **73**: 190–6.
- 185 Heuer, H., Schmitt, H., and Smalla, K. (2011) Antibiotic resistance gene spread due to  
186 manure application on agricultural fields. *Curr. Opin. Microbiol.* **14**: 236–43.
- 187 Heuer, H. and Smalla, K. (2012) Plasmids foster diversification and adaptation of bacterial  
188 populations in soil. *FEMS microbiol. Rev.* **36**: 1083–104.
- 189 Magid, J., Luxhøi, J., Jensen, L.S., and Møller, J. (2006) Establishment of a long-term field  
190 trial with urban fertilizers. In *Bruun, S., Raupp, J., Pekrun, C., Oltmanns, M. and*  
191 *Köpke, U. (eds.). Is recycling of nutrients from urban areas to peri-urban organic*

- 192 farms feasible?, *ISOFAR Scientific Series, Bonn, Germany*. Verlag Dr. H. J. Köster,  
193 pp. 59–78.
- 194 Marti, R., Scott, A., Tien, Y.-C., Murray, R., Sabourin, L., Zhang, Y., and Topp, E. (2013)  
195 The impact of manure fertilization on the abundance of antibiotic-resistant bacteria  
196 and frequency of detection of antibiotic resistance genes in soil, and on vegetables at  
197 harvest. *Appl. Environ. Microbiol.* AEM.01682–13–.
- 198 Musovic, S., Dechesne, A., Sørensen, J., and Smets, B.F. (2010) Novel assay to assess  
199 permissiveness of a soil microbial community toward receipt of mobile genetic  
200 elements. *Appl. Environ. Microbiol.* **76**: 4813–8.
- 201 Newby, D.T. and Pepper, I.L. (2002) Dispersal of plasmid pJP4 in unsaturated and  
202 saturated 2,4-dichlorophenoxyacetic acid contaminated soil. *FEMS Microbiol. Ecol.*  
203 **39**: 157–64.
- 204 Nicholson, F., Chambers, B., Williams, J., and Unwin, R.. (1999) Heavy metal contents  
205 of livestock feeds and animal manures in England and Wales. *Bioresour. Technol.* **70**:  
206 23–31.
- 207 Nielsen, K.. and van Elsas, J.. (2001) Stimulatory effects of compounds present in the  
208 rhizosphere on natural transformation of *Acinetobacter* sp. BD413 in soil. *Soil Biol.*  
209 *Biochem.* **33**: 345–357.
- 210 Poulsen, P.H.B., Al-Soud, W.A., Bergmark, L., Magid, J., Hansen, L.H., and Sørensen,  
211 S.J. (2013) Effects of fertilization with urban and agricultural organic wastes in a field  
212 trial – Prokaryotic diversity investigated by pyrosequencing. *Soil Biol. Biochem.* **57**:  
213 784–793.
- 214 Poulsen, P.H.B., Magid, J., Luxhøi, J., and de Neergaard, A. (2013) Effects of fertilization  
215 with urban and agricultural organic wastes in a field trial – Waste imprint on soil  
216 microbial activity. *Soil Biol. Biochem.* **57**: 794–802.
- 217 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The  
218 SILVA ribosomal RNA gene database project: improved data processing and web-  
219 based tools. *Nucleic Acids Res.* **41**: D590–6.
- 220 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al.  
221 (2009) Introducing mothur: open-source, platform-independent, community-supported  
222 software for describing and comparing microbial communities. *Appl. Environ.*  
223 *Microbiol.* **75**: 7537–41.
- 224 Silvertown, J., Poulton, P., Johnston, E., Edwards, G., Heard, M., and Biss, P.M. (2006)  
225 The Park Grass Experiment 1856-2006: its contribution to ecology. *J. Ecol.* **94**: 801–  
226 814.

- 227 Smets, B.F., Rittmann, B.E., and Stahl, D.A. (1995) Quantification of the effect of substrate  
228 concentration on the conjugal transfer rate of the TOL plasmid in short-term batch  
229 mating experiments. *Let. Appl. Microbiol.* **21**: 167–172.
- 230 Sørensen, S.J., Bailey, M., Hansen, L.H., Kroer, N., and Wuertz, S. (2005) Studying  
231 plasmid horizontal transfer in situ: a critical review. *Nat. Rev. Microbiol.* **3**: 700–10.
- 232 Thomas, C.M. and Nielsen, K.M. (2005) Mechanisms of, and barriers to, horizontal gene  
233 transfer between bacteria. *Nat. Rev. Microbiol.* **3**: 711–21.
- 234 You, Y., Hilpert, M., and Ward, M.J. (2012) Detection of a common and persistent tet(L)-  
235 carrying plasmid in chicken-waste-impacted farm soil. *Appl. Environ. Microbiol.* **78**:  
236 3203–13.
- 237
- 238

239 Table 1: Plasmids<sup>a</sup> carried by the red fluorescent donor strain, *Pseudomonas putida*  
 240 KT2442 *DsRed::lacIq*

Plasmid	Inc-group	Type	Phenotype	Host range	References
RP4:: <i>Plac::gfp</i>	IncP-1 $\alpha$	Resistance	Tet <sup>R</sup> , Amp <sup>R</sup> , Km <sup>R</sup>	broad	(Barth and Grinter, 1977)
pIPO2tet:: <i>Plac::gfp</i>	(IncQ-mobilizer)	Cryptic	Tet <sup>R</sup>	broad	(Tauch et al., 2002)
pRO101:: <i>Plac::gfp</i>	IncP-1 $\beta$	Catabolic	Tfd <sup>+</sup> , Hg <sup>R</sup> , Tet <sup>R</sup> ,	broad	(Harker et al., 1989)

241 <sup>a</sup>Plasmids are tagged using a TN5 cassette with a constantly expressed *gfp* gene that is  
 242 *lacI* repressed in the donor strains.

243

244

245 Table 2: Origin of the recipient microbial communities

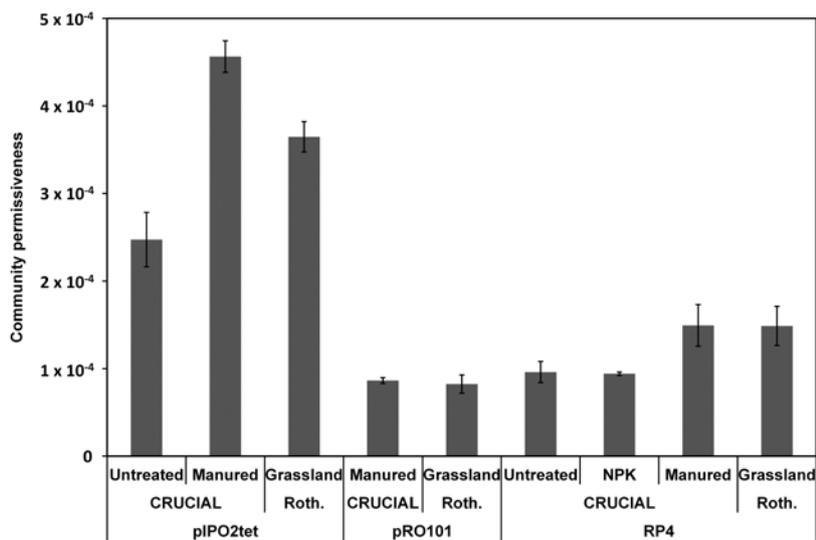
Soil origin	Soil type	Soil treatment	Coordinates	References
CRUCIAL Taastrup Denmark	agricultural	Untreated	(55.681 N, 12.276 E)	(Poulsen et al., 2013; Magid et al., 2006)
		Manured	(55.681 N, 12.276 E)	(Poulsen et al., 2013; Magid et al., 2006)
		NPK-fertilized	(55.681 N, 12.276 E)	(Poulsen et al., 2013; Magid et al., 2006)
Park Grass Rothamsted, UK	grassland	Untreated	(51.811 N, - 0.377 E)	(Silvertown et al., 2006)

246

247

248 Figure 1: Transfer frequency of the introduced plasmid to the soil indigenous bacterial  
249 community after 48 hours of incubation on filters on soil extract medium. Values are  
250 displayed as the mean of filter triplicates with standard deviation.

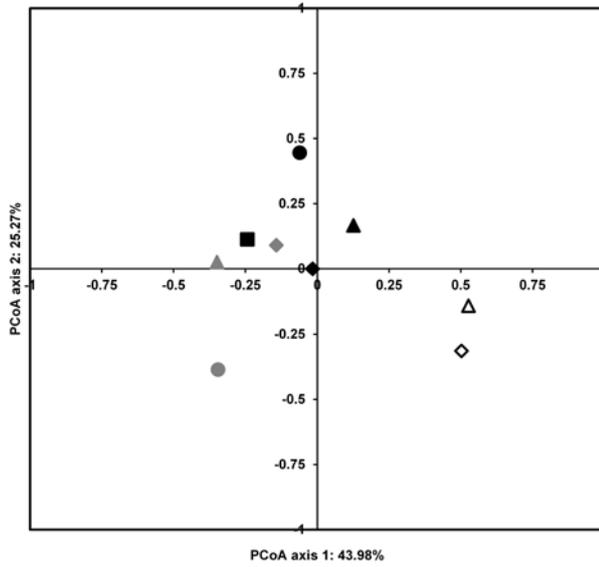
251



252

253

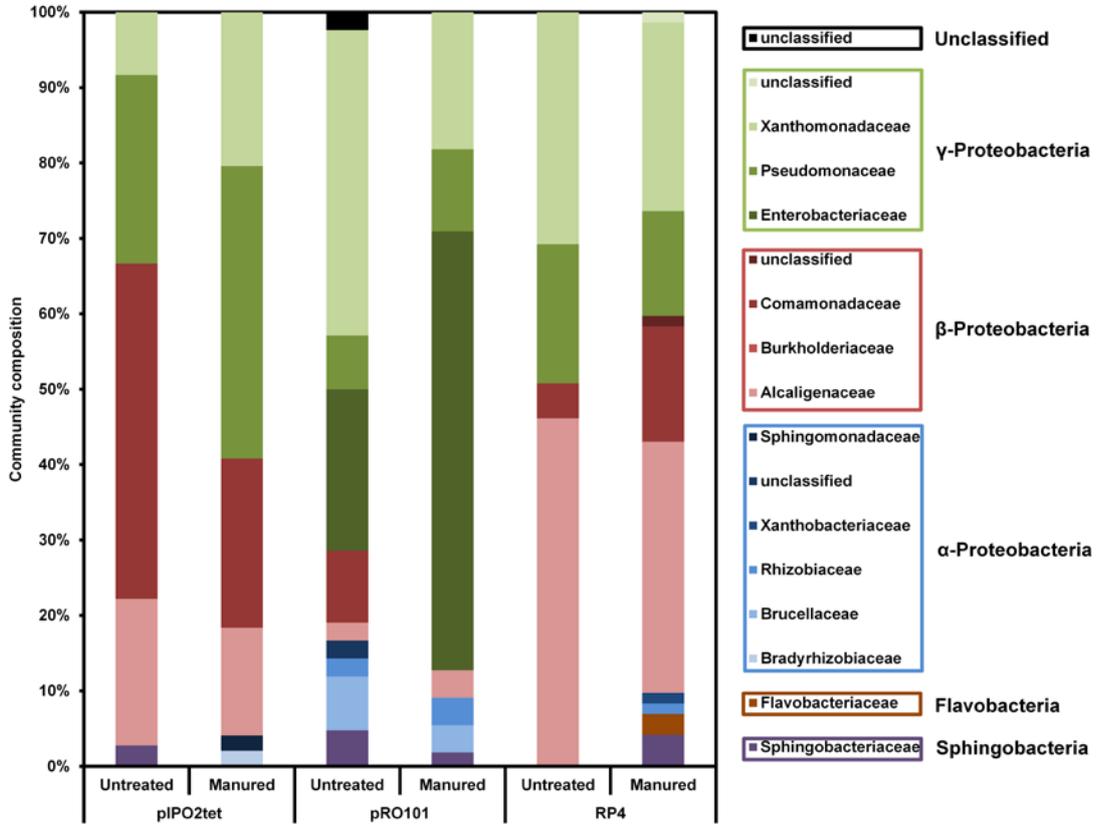
254 Figure 2: Principal Coordinate Analysis (PCoA) of the sequenced transconjugal pools.  
255 Plasmids: pIPO2tet (red), pRO101 (green), RP4 (blue); Soil microbial communities:  
256 CRUCIAL Manured (diamond), CRUCIAL Untreated (triangle), CRUCIAL NPK-fertilized  
257 (square), Rothamstaed Park Grass (circle)



258

259

260 Figure 3: Phylogenetic analysis of the transconjugal pools originating from CRUCIAL  
 261 Manured and CRUCIAL Untreated soil microbial communities for all 3 tested plasmids.



262

263

264 SI Table 1: Diversity of transconjugants sorted by OTUs and occurrence per transconjugal  
 265 pool.

OTU	Kingdom	Phylum	Class	Order	Family	Genus	pIPOZtet				pRO101		RP4			total sequences
							Untreated	CRUCIAL	NPK	Manured	Rothamstead	Park Grass	Untreated	Manured	CRUCIAL	
1			Flavobacteria	Flavobacteriales	Flavobacteriaceae	Chryseobacterium	0	0	0	0	0	0	0	1	0	1
2						unclassified	0	0	0	0	0	0	1	0	1	
3						Pedobacter	0	0	0	0	0	0	2	1	3	
4		Bacteroidetes					1	0	0	0	0	0	1	0	2	
5			Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	0	0	0	0	1	0	0	0	1	
6						unclassified	0	0	0	0	0	1	0	0	1	
7						unclassified	0	0	0	0	1	0	0	0	1	
8					Bradyrhizobiaceae	Bosea	0	0	1	0	0	0	0	0	1	
9					Bruceellaceae	Ochrobactrum	0	0	0	1	3	0	0	0	4	
10				Rhizobiales		unclassified	0	0	0	0	0	2	0	0	2	
11		Alphaproteobacteria			Rhizobiaceae	Rhizobium	0	0	0	1	1	2	0	1	5	
12					unclassified	unclassified	0	0	0	0	1	0	0	0	1	
13					Xanthobacteraceae	unclassified	0	0	0	0	0	0	0	1	1	
14				Sphingomonadales	Sphingomonadaceae	Sphingomonas	0	0	1	0	0	0	0	0	1	
15						unclassified	7	9	7	2	1	2	27	21	4	80
16					Alcaligenaceae	Achromobacter	0	0	0	0	0	0	1	1	0	2
17					unclassified	unclassified	0	0	0	0	0	0	0	1	0	1
18					unclassified	unclassified	0	0	0	0	0	0	1	0	1	
19		Betaproteobacteria		Burkholderiales		unknown	0	0	0	0	0	0	1	1	3	
20					Burkholderiaceae	unknown	0	0	0	0	0	0	0	0	5	5
21					Comamonadaceae	Variovorax	16	0	11	0	4	0	3	11	0	45
22					unclassified	unclassified	0	0	0	0	0	0	0	1	0	1
23					Pantoea	Pantoea	0	0	0	0	1	0	0	0	0	1
24	Bacteria					unclassified	0	0	0	0	8	25	0	0	0	33
25						unclassified	0	0	0	0	0	2	0	0	0	2
26		Proteobacteria		Enterobacteriales	Enterobacteriaceae	unclassified	0	0	0	0	0	1	0	0	0	1
27						unclassified	0	0	0	0	0	1	0	0	0	1
28						unknown	0	0	0	0	0	3	0	0	0	3
29						unclassified	8	4	18	1	2	5	11	10	2	61
30						unclassified	0	0	0	0	1	0	0	0	0	1
31						unclassified	0	0	0	0	0	1	0	0	0	1
32					Pseudomonas	Pseudomonas	0	0	0	0	0	0	1	0	0	1
33				Pseudomonadales	Pseudomonadaceae	unclassified	0	1	0	0	0	0	0	0	0	1
34		Gammaproteobacteria				unclassified	0	0	1	0	0	0	0	0	0	1
35						unclassified	0	0	0	1	0	0	0	0	0	1
36						unclassified	1	0	0	0	0	0	0	0	0	1
37						unclassified	0	3	4	0	1	0	18	7	10	43
38						unclassified	3	4	6	0	6	8	1	11	3	42
39						unclassified	0	0	0	0	4	0	0	0	0	4
40					Stenotrophomonas	Stenotrophomonas	0	0	0	0	0	2	0	0	0	2
41				Xanthomonadales	Xanthomonadaceae	unclassified	0	0	0	0	1	0	0	0	0	1
42						unclassified	0	0	0	0	0	0	1	0	0	1
43						unclassified	0	0	0	0	4	0	0	0	0	4
44						unclassified	0	0	0	0	1	0	0	0	0	1
45			unclassified	unclassified	unclassified	unclassified	0	0	0	0	0	0	0	1	0	1
46			unclassified	unclassified	unclassified	unclassified	0	0	0	0	1	0	0	0	0	1
						<b>total OTUs</b>	<b>6</b>	<b>5</b>	<b>8</b>	<b>5</b>	<b>18</b>	<b>13</b>	<b>10</b>	<b>16</b>	<b>7</b>	<b>46</b>
						<b>total sequences</b>	<b>36</b>	<b>21</b>	<b>49</b>	<b>6</b>	<b>42</b>	<b>55</b>	<b>65</b>	<b>72</b>	<b>26</b>	<b>372</b>

SI Table 1: Diversity of transconjugants sorted by OTUs and occurrence per transconjugal pool. Sequences were analyzed using mothur v1.30.0 (Schloss et al., 2009) and the SILVA database (Quast et al., 2013). These sequence data have been submitted to the GenBank database under submission ID 1656830.

266

267

268 SI 2: Sampling procedure and information

269 Soil samples of three different treatments were taken at the annually manured CRUCIAL  
270 (Closing the Rural Urban Nutrient Cycle) agricultural field site (Taastrup, Denmark) (Magid  
271 et al., 2006; Poulsen et al., 2013). Soil samples were collected in late fall 2010. Samples of  
272 each treatment were taken from three different plots of this treatment. Each plot was  
273 sampled for 1 kg of soil at 15 locations. The resulting soil volume was sieved and  
274 homogenized to obtain a representative sample. Twenty grams of this homogenized soil  
275 samples were used for Nycodenz®-extraction. The fourth sample was taken from the  
276 monitored untreated Park Grass field in Rothamsted (Silvertown et al., 2006) (Rothamsted,  
277 UK) in the exact same manner.

278