UNIVERSITÀ DEGLI STUDI DI SASSARI

SCUOLA DI DOTTORATO DI RICERCA
Scienze e Biotecnologie
dei Sistemi Agrari e Forestali
e delle Produzioni Alimentari

Indirizzo: Produttività delle piante coltivate

Ciclo XXVIII

METAGENOMIC ANALYSIS OF BACTERIAL ASSEMBLAGES FROM SARDINIAN SOILS

Dott.ssa Sanna Monica

Direttore della Scuola Prof. Antonello Cannas

Referente di Indirizzo Prof.ssa Rosella Motzo

Docente Guida Prof. Andrea Porceddu

Anno accademico 2014- 2015

UNIVERSITÀ DEGLI STUDI DI SASSARI

SCUOLA DI DOTTORATO DI RICERCA
Scienze e Biotecnologie
dei Sistemi Agrari e Forestali
e delle Produzioni Alimentari

Indirizzo: Produttività delle piante coltivate

Ciclo XXVIII

La presente tesi è stata prodotta durante la frequenza del corso di dottorato in "Scienze e Biotecnologie dei Sistemi Agrari e Forestali e delle Produzioni Alimentari" dell'Università degli Studi di Sassari, a.a. 2014/2015 - XXVIII ciclo, con il supporto di una borsa di studio finanziata con le risorse del P.O.R. SARDEGNA F.S.E. 2007-2013 - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linea di Attività l.3.1 "Finanziamento di corsi di dottorato finalizzati alla formazione di capitale umano altamente specializzato, in particolare per i settori dell'ICT, delle nanotecnologie e delle biotecnologie, dell'energia e dello sviluppo sostenibile, dell'agroalimentare e dei materiali tradizionali".

Monica Sanna gratefully acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007-2013 - Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.)

Anno accademico 2014- 2015

Index

Introduction

The Soil Microbiota

The soil can be considered as the living skin of the Earth (Pepper et al., 2009): an highly heterogeneous environment populated by a complex microbial community (Daniel, 2005).

Soil is a structured, heterogeneous and discontinuous system; it is generally poor in energy sources, nutrients and the different components of its solid fractions as organic matter, clay, sand and silt content form various microhabitats. The chemical, physical and biological properties of these microhabitats differ in time and space (Nannipieri et al., 2003) allowing the survival of an enormous biodiversity (Torsvik et al., 1996; Rosello-Mora and Amann, 2001).

The soil biota can be classified in:

- microflora (1-100 μm, essentially bacteria and fungi),
- microfauna (5-120 μm, such as protozoa, nematodes and some mites that feed mainly preying bacteria),
- mesofauna (0,08-2 mm, such as springtails and mites that feed mainly on small insects and fungal mycelia),
- macrofauna (500 μm-50 mm, such as worms and termites),
- megafauna (organism >20 mm as insects, mammals and especially earthworms) (Swift and al., 1979).

The number of microorganisms varies according to the ecosystem from few thousand of species per gram of sand dune soil to several hundred thousand depending also on the temperature and moisture, the physical state and depth of the soil. The fauna plays a crucial role in the dissemination of microorganisms. Animals disseminate other microorganisms that are attached to their body surface. The nematodes are known for the capacity of dissemination especially of the bacteria in the soil. Anderson et al. (2003) reported that the nematode *Caenorhabditis elegans* has the ability to disseminate bacteria with preference for Gram-neagative bacteria compared to Gram-positive. The excrement of invertebrates appears as outbreaks from which the bacteria can spread in soil environments (Giardini, 2004). Especially protozoa and nematodes ingest other microorganisms and thus contribute with their excrement to the spread and maintenance of biological diversity (Anderson et al., 2003). This action is crucial to avoid the local concentration of some groups of

microorganisms, a matter that is particularly important for example for bacteria that are most sensitive to grazing (Wardle and Lavelle, 1997). In fact, soil protozoan activity is restricted to water films and water-filled pores, and small pores may protect bacteria from grazing.

The protozoan populations are found at high proportions in saturated soils while the lowest proportion was reported to dry soils (Darbyshire, 1976).

Postma et al. (1989) observed an increased survival rate of bacterial cells introduced into relatively dry soils in comparison to those inoculated into wetter soils, and suggested that protozoan predation as a possible cause.

The heterogeneous and discontinuous structure of soil provides a number of distinct or temporally discrete microhabitats, which are influenced by environmental fluctuations (Hattori and Hattori, 1976).

Alexander (1981) proposed the importance of such microsites in the survival of bacterial inoculates where they exclude the predator and protect the prey.
The predatory activities of the protozoa under such conditions are thought to be restricted, since they cannot access to their prey due to their larger size.

Soil microorganisms can degrade a variety of organic compounds of anthropogenic origin so contributing to the recovery of endangered habitats (Winding et al., 2005). The degradation and mineralization of organic complexes, the recycling of carbon, nitrogen, phosphorus, and sulfur are profoundly affected by soil microorganisms. Pesticides, fertilizers, and other agriculture practices have significant effects on the structure of microbial communities as well as on the chemical and physical properties of the soils (Garbeva et al., 2004). Many studies have recently reported how heavy metals may affect the microorganism diversity by acting adversely on their growth, biochemical activity (Sandaa et al., 2001). Wang et al. (2007) have demonstrated that the diversity and activity of the microbial communities are negatively correlated to the extractable fraction of heavy metals in contaminated soils. Since most of these heavy metals derived from industrial activities and can contaminate not only the land but also the waters, soil microbial diversity and activity were proposed as an indicator of the level of environmental hazard deriving from human activities (McGrath et al., 2001; Shi et al., 2002).

Among the physical factors able to influence the edaphic community, temperature that acts directly on the microbial metabolism, moisture and light (Lambers, 1998) are expected to play a crucial role in regulating microbiota activities and diversity. In conditions of low light intensity, metabolic activity and respiration of roots can be reduced by up to 50%

(Lambers, 1987) and this obviously affects microbial activity, as the carbonaceous substrates become limiting (Broughton and Gross, 2000).

The taxonomic composition of the bacterial fractions of soil has been recently investigated by employing the so called "omic" approaches. Deep sequencing of 16S rRNA based libraries have demonstrated an enormous diversities of bacteria inhabiting the soils. The analysis of literature indicated that some phyla are common to all investigated soils while other are specific of particular soil microhabitats. The most represented phyla are: *Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Cloroflexi, Planctomycetes, Gemmatinomodes* and *Firmicutes*. Sequences deriving from species belonging to these nine phyla, on average, make up more than 90% of soil libraries. For a description of the relative average contribution of each phylum see figure 1.

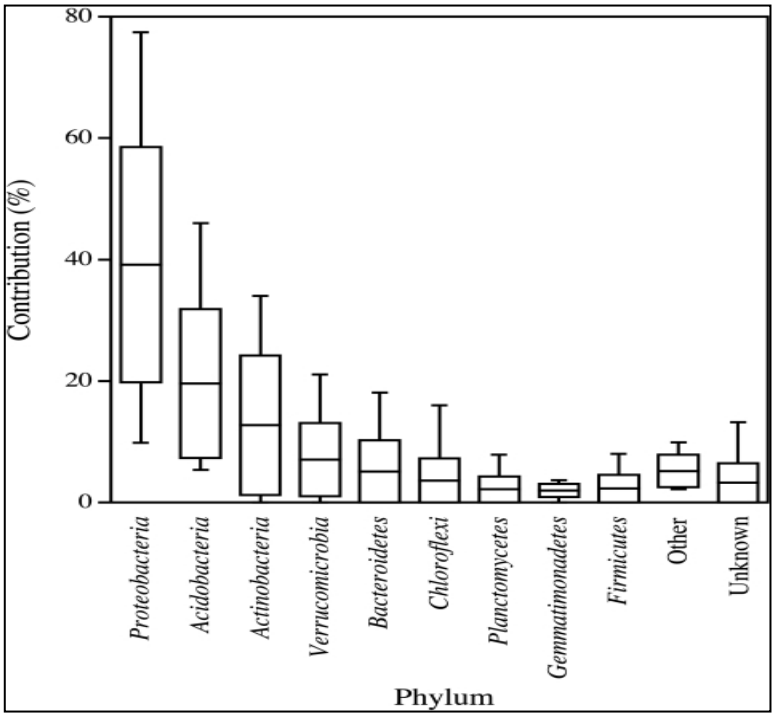

Figure 1: Contribution of 16S rRNA and 16S rRNA genes from members of different phyla (Janssen, 2006)

In general, the phylum *Proteobacteria* and *Actinobacteria* are the most represented in soils as all libraries surveyed to date reported sequences that are assigned to these two phyla.

Table 1 reports the estimates of members of different bacterial groups in 16s rRNA libraries constructed from genomic DNA extracted from different soil samples. *Alphaproteobacteria, Acidobacteria* and *Actinobacteria* are often more abundant in soils than members of *Bacteroidetes, Firmicutes* and *Planctomycetes*.

Phylum	Subphylum	Mean contribution (%)	Range (%)
Acidobacteria	Subdivision 1	3.3	0–14.4
	Subdivision 2	0.5	0–3.3
	Subdivision 3	1.8	0–4.9
	Subdivision 4	7.7	0–35.0
	Subdivision 5	0.4	0–2.2
	Subdivision 6	4.5	0–12.8
	Subdivision 7	1.5	0–7.4
Actinobacteria	*Acidimicrobidae*	2.4	0–8.9
	Actinobacteridae	4.7	0–18.3
	Rubrobacteridae	5.6	0–24.8
Proteobacteria	*Alphaproteobacteria*	18.8	1.8–43.1
	Betaproteobacteria	10.0	2.1–31.1
	Gammaproteobacteria	8.1	1.1–34.1
	Deltaproteobacteria	2.3	0–10.1
	Epsilonproteobacteria	0.04	0–0.8
Verrucomicrobia	*Verrucomicrobiae*	0.03	0–0.7
	Spartobacteria	6.3	0–21.1
	Subdivision 3	0.5	0–4.7
	Subdivision 4	0.2	0–1.1
Bacteroidetes	*Flavobacteria*	0.4	0–3.2
	Sphingobacteria	4.6	0–15.9
Firmicutes	*Bacilli*	1.6	0–7.0
	Clostridia	0.2	0–1.4
Chloroflexi		3.2	0–15.8
Planctomycetes		2.0	0–7.8
Gemmatimonadetes		2.0	0–3.7
Other groups		5.2	2.2–9.9
Unknown		2.4	0–12.6

Table 1: Contribution of 16S rRNA and 16S rRNA genes in Janssen rewiew (2006)

However, it is important to note that the relative abundance of major phyla varies significantly between different soils. For example the study of Felske et al. (1998) has identified *Firmicutes* as the major phylum in chalk soils of the Netherlands, although this phylum is always below 10% in other surveyed libraries. Similar findings have been reported by Teixeira et al. (2010) and Kuramae et al. (2012).

Members of *Proteobacteria* phylum make up an average of 39% of libraries. Most soil-dwelling, *Proteobacteria* are classified within the *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria* and *Deltaproteobacteria* classes.

Members of the *Acidobacteria* phylum make up an average of 20% of soil communities (Naether et al., 2012). The *Acidobacteria* phylum is divided into at least eight classes/subdivisions. Three of these, subdivisions 1, 4 and 6, are particularly abundant in

soils. Members of subdivision 6 may be aerobes, since they were not detected in permanently anoxic soil systems (Naether et al., 2012). The phylogenetic depth of the phylum *Acidobacteria* is particularly high, with some subdivisions including mostly aerobic isolates (1 to 4) while other being obliged anaerobes.

The *Actinobacteria* phylum is composed of three subclasses that are common in soil: *Actinobacteridae* (the most present), *Acidimicrobiae* and *Rubrobacteridae* (Janssen et al., 2006).

Among the characterized *Acidimicrobiae, Acidimicrobium ferroxidans,* and *Ferromicrobium acidophilus* are worth mentioning (Garrity et al., 2004). These are ferrous-iron-oxidizing acidophylus and *Micothrix parvicella*, a filamentous bacterium. The genes *Solirubrobacter* is the least characterized aerobic heterotrophs in the subclasses *Rubrobacteridae* (Janssen et al., 2006).

The phylogenetic depth of the phylum *Actinobacteria* is lower than that of *Proteobacteria* and *Acidobacteria*, although this phylum has a considerable high level of phenotypic diversity.

The *Verrucomicrobia* are divided into five major classes (Janssen et al., 2006). The major group of *Verrucomicrobia* found in soil is the class *Spartobacteria*. The phylum *Bacteroidetes* make up an average 5% (range 0-18 %) of soil bacteria (Sangwan et al., 2006) Some members of this group are aerobes, while others are anaerobes or facultative anaerobes and thus species composition of members of this class within a soil may be used to infer oxygen levels availability in soil.

The phylum *Cloroflexi* makes up an average of 3% of soil communities (Janssen et al., 2006), consists of 8 classes and has a phylogenetic depth comparable to *Proteobacteria*. The *Planctomycetes* represent on average 2% of bacterial communities. These are budding bacteria which can be classified in three classes. Most isolates of this phylum are from aquatic resources and it is in doubt if these are physiologically and genetically suitable models for soil *Planctomycetes* (Fuerst, 2005).

The *Gemmatimonadetes* phylum contains only one described species, *Gemmatimonas aurantiaca*, a gram-negative aerobic heterotrophic. The diversity of general physiology of this group is well defined by Zhang et al. (2003)

The phylum *Firmicutes* consists of several classes with *Bacillus* and *Clostridium* being the most important. It is possible that members of this group are not adequately represented in 16s rRNA libraries because cells or spores are particularly though and may be

difficult to lyse (Janssen et al., 2006; Kuramae et al., 2012).

To date much research effort has been dedicated at understanding how agriculture and land management functions influences microbial community. Several studies have attempted at identifying the effect of agricultural practices or of physical-chemical properties on soil bacteria taxonomic composition and activity. Here we summarize some of the findings which have been confirmed by several studies or that describe effects that seem to have a general validity (see table 2).

Effect of grazing

Bardgett et al. (2001) have examined the effects of grazing to soil microbial diversity and activity. These authors have analyzed several sites in UK where long-term variations in the frequency and intensity of grazing by sheeps has led to the establishment of successional transitions, from ancient and unmanaged oak (*Quercus petraea*) woodland, which is essentially not grazed, to heavily grazed grassland where more than 90% of the annual aboveground productivity (AAP) is consumed by sheeps.

The successional transitions induced modifications in the total microbial communities that relate to both direct physical effects of herbivores (Nicholson et al., 1970; Grant et al., 1985) and to indirect positive effect of herbivores on ecosystem productivity. The indirect effects were reconducted to a more efficient recirculation of nutrients via animal excreta pathway (Floate et al.,1971 a/b; Ruess and McNaugthon, 1987) and in some cases to improvements in plant litter quality and decomposability of grazed plants.

Accelerated nutrient cycling in grazed grassland may also be associated to an increase in soil carbon supply which may have affected microbial biomass activity. Grazing was also reported to have a beneficial effect on mineralization with positive consequences on soil nutrient availability and greater shoot nutrient content and productivity.

The authors reported that the C/N ratio increased along the gradient of grazing intensity. There was no consistent trend in microbial activity as measured by basal respiratory along the gradient of grazing. Interestingly, the highest level of respiration rate was registered for the lightly grazed grassland. The microbial biomass, measured as PLFA (phospholipid fatty acid) varied significantly along the gradients and was the highest in the lightly grazed grassland but no gradient associated to the grazing intensity was observed. The soil pH depicted a gradient that was positively associated with grazing intensity.

		No. of members of indicated group[c]											
Source (site designation)[a]	Method[b]	ACI	ACT	ALF	BET	GAM	DEL	VER	BAC	FIR	PLA	PRO	Reference[d]
Cropland, Italy	FISH		4	20	15	5			8	6	5		1
Organic soil, Norway	FISH		<1	2-5	<1-5	<1	5-8		<1		3-7		1
Mineral soil, Germany	FISH		<1	7-10	<1-4	<1	4-7		<1		3-7		1
Tundra, Russia	FISH		4	1	12	3			8	6			1
Cropland, Germany	FISH			3	10	25			2				1
Forest, Germany	FISH		<1	7	<1	1	3		<1		7		1
Tilled cropland, Unites States	rRNA	<1-9	5-22	18-41	2-6			<1-3	<1		5-7		1
Tilled cropland, Unites States	rRNA		7	26	4	3							1
No-till cropland, Unites States	rRNA		7	26	4	3							1
No-till cropland, Unites States	rRNA		11	31	5	3							1
Abandoned field, Unites States	rRNA	1-3	9-27	7-33	1-5			1-3	1-3		3-7		1
Abandoned field, Unites States	rRNA	4	10	38	1			2	2		13		1
Tilled grassland, Unites States	rRNA	2	14	4	<1			<1	<1		2		1
Meadow, Unites States	rRNA	<1-3	9-18	12-41	1-9	4		1-3	1-3		4-12		1
Tree plantation, Unites States	rRNA	1	7	16	1			<1	<1		3		1
Meadow, The Netherlands	rRNA		19	22						48			1
Desert, Unites States	qPCR	19	5	7	4					4			1
Forest, Unites States	qPCR	14	5	14	5					3			1
Prairie, Unites States	qPCR	23	6	9	8					6			1
Spruce age class forests *	rRNA	22	9	42	3	4	1	<1	<1	<1	<1	3	2
Spruce age class forests*	rRNA	23	11	33	2	1	1	<1	<1	<1	<1	11	2
Spruce age class forests*	rRNA	23	15	18	5	1	1	<1	<1	<1	<1	12	2
Beech age class forests*	rRNA	20	13	14	8	3	4	<1	<1	<1	<1	6	2
Beech age class forests*	rRNA	19	12	22	6	2	7	<1	<1	<1	<1	6	2
Beech age class forests*	rRNA	20	13	19	5	2	5	<1	<1	<1	<1	6	2
Unmanaged beech forests*	rRNA	15	14	28	5	2	6	<1	<1	1	<1	5	2
Unmanaged beech forests*	rRNA	15	14	26	8	3	5	<1	<1	<1	<1	7	2
Unmanaged beech forests*	rRNA	21	9	19	7	4	6	<1	<1	<1	<1	9	2
Cultivates, Unites States	rRNA	4	33	22	6		8	3	4	2	2	40	3
Forested, Unites States	rRNA	20	20	33	2		3			4	3	41	3
Pastured, Unites States	rRNA	8	34	31	4		8				2	45	3

Table 2: Relative abundance (%) of bacteria phyla in different soils

a The site designations are those used by authors to identify particular sources within studies with multiple soil samples.

b FISH, counting of cells in soil samples with group-specific oligonucleotide probes; rRNA, estimation of abundance of rRNA in total rRNA by hybridization with group-specific oligonucleotide probes; qPCR, quantitative PCR estimate of 16S rRNA genes using group-specific assays, relative to estimates of total bacteria using Bacteria-specific assays.

c ACI, phylum *Acidobacteria*; ACT, phylum *Actinobacteria*; ALF, class *Alphaproteobacteria*; BET, class *Betaproteobacteria*; GAM, class *Gammaproteobacteria*; DEL, class *Deltaproteobacteria*; VER, phylum *Verrucomicrobia*; BAC, phylum *Bacteroidetes*; FIR, phylum *Firmicutes*; PLA, phylum *Planctomycetes*; PRO, phylum *Proteobacteria*; WS3 division *Wurtsmith* contamined aquifer.

d 1 (Janssen, 2006); 2 (Nacke et all, 2011); 3 (Shange et all, 2012).

*Germany.

The microbial diversity and evenness was maximum in ungrazed soil suggesting that intensive grazing select dominant group of species. The evenness reduction was found associated to an increase of soil pH. The fungi/bacteria ratio was negatively related to grazing activity, supporting the notion that intensively grazed or disturbed ecosystems have decomposition channels that are bacterial based, and that fungi are relatively more important in decomposer food webs of less disturbed systems.

Effect of pH on soil microbial diversity and activity

Several studies have indicated that pH is a reliable predictor of the soil bacterial diversity at level of phylum. Interestingly, soil pH was reported also as a predictor of variability in diversity level within each of the five common phyla. *Acidobacteria, Actinobacteria* and *Bacteroidetes* are generally strongly correlated with soil pH. However, the analysis of the specific mechanisms responsible for the relations between soil microbial diversity and pH has not yet produced a consensus view. Lauber et al. (2009) have advanced two general hypotheses which are not mutually exclusive. First, soil pH may influence microbial community by an indirect mechanism. A number of soil characteristics such as cationic metal solubility, organic C characteristic, soil moisture etc., are often related to soil pH and these factors may drive the observed changes in community composition. The relations between soil pH and microbial community composition may be indirect and mediated by one or more of these soil variables.

The second hypothesis takes into consideration that pH directly imposes a physiological containment of soil bacteria, altering competitive outcomes or reducing the net growth of individual taxa.

Effect of different land management

Shange et al. (2012) have analyzed the effects of three different land use systems on the taxonomic composition of soil microbiota in the United States.

The three management systems were: pine forest that was grazed by sheeps, cultivated crop and grazed pasture. The experimental approach was based on deep sequencing of a 16S rRNA library. The grazed pasture showed the highest species diversity probably as result of an input of bacteria with fecal and urin deposition. Such an effect on bacterial diversity was however mitigated by the effect of acidic pH of forest sites that caused a significant reduction of microbial activity and diversity. The cultivated land showed the highest species

evenness. The most abundant groups were: *Deltaproteobacteria, Betaproteobacteria, Alphaproteobacteria, Verrucomicrobia, Planctomycetes, Gemmatimonadetes, Firmicutes* and *Proteobacteria.* This latter group represents about 40% of total bacteria species in all sites. The *Acidobacteria* represent 20,6% of total bacteria of forest soil and only 8,6% of total bacteria of cultivated land.

Nacke et al. (2011) have reported the results of a similar study applied to different soils of Germany that were subjected to six different management systems. The sites were sampled by four forested sites: one with pine, spruce age or beech age and another with unmanaged beech. Other sites were located in pastures grazed by horses or cattles and unfertilized pasture grazed by sheeps. Finally, the last sites were sampled in intensively cultivated grassland. The most striking difference was observed for the sites sampled in the forest, a finding that could be explained by the effect of acidic pH on microbial diversity and activity. The predominant phyla in forest soil were *Acidobacteria* while *Proteobacteria* dominated the other soils. The only relevant feature of grazed soil was an increase of species belonging to the class *Firmicutes*.

Kuramae et al. (2012) have reported the results of an analysis of microbial diversity carried out on twenty-five fields which represent six of the most important land use in the Netherlands. The samples were analyzed by denaturing gradient gel electrophoresis (DGGE) and by hybridization to a PhyloChip. The DGGE analysis showed no clear separation of the fields according to land use. The most separated samples were those coming from forested sites. PhyloChip analysis demonstrated that arable soils, natural grasslands, pasture and deciduous forest soils had 38% to 42% more OTUs than forests soils. Firmicutes was the most represented phylum.

Effect of pollution and heavy metal in microorganism of soil

Metals are essential components of the soil ecosystem, whose biologically available concentrations depend mainly on geological and biological processes (Ehrlich, 2002). There are several definitions of heavy metals, and some of them are based on the mass density of these elements. Within the group of heavy metals one can distinguish both elements that are essential for living organisms (microelements) and the elements whose physiological role is unknown and thus they are “inactive” towards organisms. The metals that serve as microelements in living organisms usually occur in trace amounts, precisely defined for each species and both their deficiency and excess badly affect living organisms. The effect of any

substance on a living system is always dependent on its available concentration to cells. Also, several heavy metal ions are crucial in metabolic processes at low concentrations but are toxic at high concentrations. Heavy metal contaminants in the environment are deposited in soils in some form of a low solubility compound, such as pyrite on surface-reactive phases, such as Fe and Mn oxides (Huerta-Diaz, 1992). While this phenomenon immobilizes the contaminants, thus limiting their effects upon biota and human health, it also places metal ions in an intimate contact with soil microbial community.

The knowledge of metal effects at lower, more environmentally relevant conditions, pH, and response of metagenome to heavy metal challenge under these conditions is still limited to a handful of studies.

Although some heavy metals are required for physiological life's processes (components of metal enzymes), their excessive accumulation in living organisms is always detrimental. Generally, toxic metals cause enzymes inactivation, damage cells by acting as antimetabolites or form precipitates or chelates with essential metabolites (Forstner, 1995).

Pb is a common environmental contaminant found in soils and unlike other metals, Pb has not biological role, and it is potentially toxic to microrganisms (Sobolev and Begonia, 2008). The effects of low (1 ppm) and high (500–2000 ppm) levels of lead (Pb) upon the soil microbial community were investigated by analysis of the 16S and nirK gene markers, wich are indicative of general microbial and denitrifying communities, respectively. The results indicated that Pb has detectable effects upon the community diversity even at the lowest concentration tested. More interestingly preliminary data obtained in this study suggest that the denitrifying microbial community adapts to elevated levels of Pb by selecting for metal-resistant forms of nitrite reductases.

Oliveira and Pampulha (2006) have conducted a study, on the total heavy metal content and effects on soil microbiological characteristics in area with known long-term pollution problems. The total heavy metal concentrations of contaminated soil samples were 109 mg/kg and 1558 mg/kg for Hg and As, respectively. Key microbiological parameters measured were dehydrogenase activity, ATP content and number of culturable aerobic bacteria, *Actinomycetes*, fungi and symbiotic nitrogen-fixers. Quantitative analysis of soil microbial populations showed a marked decrease in total culturable numbers of the different microbial groups of the contaminated soil samples. Moreover their results suggested a good relationship between acid DHA (Docosahexaenoic acidinhibition) and heavy metal contamination. Additional microbiological properties were also evaluated, such as microbial

counts (heterotrophic aerobic bacteria, actinomycetes, fungi and asymbiotic nitrogen-fixers) and ATP content, as indicators of soil microbial biomass were also valuated. The total number of cfu of bacteria, fungi and actinomycetes were significantly reduced in the contaminated sites. However, fungi and actinomycetes seemed to be less sensitive than culturable heterotrophic bacteria or even asymbiotic nitrogen fixers.

Effect of forest on the bacteria in the soil

In forest soils, bacteria occur in largest abundance in the uppermost layers the organic (O) and the accumulation (A) horizon (Raubuch and Beese, 1995). Bacteria inhabiting the uppermost soil horizons are exposed to various external stressors with the long periods of drought followed by rapid rewetting being the most common ones (Schimel et al., 2007). Several climate models forecast more frequent and longer periods of drought in multiple forested regions of the world (IPCC 2007). Thus, drought and rewetting stress will likely become a more important perturbation to forest biogeochemical cycling in many regions (Maracchi et al., 2005).

Different groups of soil bacteria may have different vulnerability to drought and rewetting stress depending on their copiotrophic or oligotrophic character (Fierer et al., 2007) or their desiccation-related life-strategies.

For example, Chodak et al. (2010) have conducted experiments to test the reaction of different bacterial phyla to drought and rewetting stress and to investigate how soil properties influence the reaction of different bacterial phyla to drought and rewetting.
Their study showed that prior to the stress, the dominating bacterial phylum was *Proteobacteria*, which constituted up to 57,5% of the total OTUs detected. Among *Proteobacteria* the most abundant was the *Alphaproteobacteria* class, followed by *Gammaproteobacteria* and *Betaproteobacteria*. Large shares were found also for *Acidobacteria* (up to 34,6% of the total OTUs detected), *Actinobacteria* (up to 15,2% of the total OTUs detected), *Bacteroidetes* (up to 7,0% of the total OTUs detected) and *Planctomycetes* (up to 4,2% of the total OTU's detected).

After the drought and rewetting stress, the average share of *Proteobacteria* phylum decreased significantly in different classes. For example the class of *Gammaproteobacteria* decreased from 15,9% to 10%.

Negative effect of the drought and rewetting stress was observed for *Bacteroidetes* and bacteria classified as "others", representing less abundant taxons. The shares of

Chloroflexi, Gemmatimonadetes and *Verrucomicrobia* were negatively affected by drought and rewetting stress, although abundances of these bacterial phyla depended mainly on soil pH. After the drought and rewetting stress increase of *Firmicutes* and *Actinobacteria* were observed.

Soil metagenomics

The soil microbiota is investigated by technical approaches that are usually grouped in two main categories i) cultivation-based and ii) cultivation independent techniques. Only 0,1 to 1% of known soil bacteria species are today "culturable".

Most of soil species are not tractable using conventional cultivation methods and therefore these must be studied by cultivation independent techniques.

Non cultivation based techniques

To circumvent some of the limitations of cultivation based approaches, indirect molecular methods based on the isolation, amplification and sequencing of nucleic acids extracted from soil samples have been developed. The microbial DNA isolated from a soil sample represents a collection of genomic DNA of all organisms inhabiting that soil and therefore should be considered as the "soil metagenome" (Daniel, 2005). Because there are no available techniques to efficiently separate DNA molecules in fractions representing each single species, the (meta)genomic DNA is analyzed without any prior separation of the composing genomes. The species contributing with their genomes to the metagenome are then identified based on phylogenetic analysis of sequences that are isolated or amplified from the whole metagenomic template or library (Daniel, 2005; Janssen, 2006; Sholz et al., 2012).

Phylogenetic surveys can be carried out by PCR amplification of 16S rRNA genes from soil DNA or using universal primers for bacteria and/or archea. These surveys allow cataloguing and comparison of the microbial diversity in different soil microhabitats and the comparative analysis of changes in community structure. The sequences of the HSP70 type chaperone gene (dnaK) and of the ammonia monooxygenase A (amoA) have been employed to survey the soil metagenome (Yap et al., 1996; Webster et al., 2002).

Analysis of soil metagenome by construction of soil DNA libraries

The construction of genomic libraries of soil metagenome can be obtained with the same methods that are suitable for constructing genomic libraries of a single species. Although these methods are conceptually simple, the complexity of the metagenome and the extremely large number of clones that would be required for full coverage of soil metagenome make this a very challenging task (Daniel, 2005; Shokralla et al., 2012).

A major breakthrough in soil metagenomics was the construction and screening of these libraries by functional or sequence based approaches. Novel genes that encode useful enzymes were identified and characterized by direct cloning from soil DNA or screening of genomic libraries. In some cases the cloned genes had little sequence homology to known genes a finding that underlines the gene discovery potential of soil borne libraries (Lorenz, 2002). Among the factors that may influence the representativeness of soil metagenomic DNA, the extraction procedure is believed of crucial importance.

Procedure of DNA extraction from soil

As soils are heterogeneous systems, details or physical properties such as particle size, soil type, moisture, pH may all influence DNA extraction efficiency. Indeed, some of the procedures for DNA purification/extraction may co-extract humic substances which could interfere with subsequent DNA modification such as restriction based digestion or PCR amplification (Daniel, 2005).

Based on the step at wich cell lysis is realized, DNA extraction methods can be divided in two categories: i) direct lysis of all cells (without any prior separation) contained in the sample (Ogram et al., 1987), and ii) extraction of cells and subsequent lysis. The amount of DNA extracted from different soil types using a selection of protocols ranges from 1 μg to 500 μg of DNA per gram of soil. To achieve direct cell lysis a combination of enzymatic and detergent-based treatments have been employed. Mechanical disruption such as bead-beating freeze-thawing or grinding of samples were also employed to lyse cells. Eukaryotic DNA from fungi, plants insects etc is usually co-extracted with these procedures and thus the metagenome represented in the DNA sample is highly heterogeneous.

Methods based on previous separation of cells, although less efficient in terms of amount of DNA are less harsh than direct lysis (Holben et al., 1998). As a consequence, the recovered DNA is on average of larger size, better quality and thus more suitable for library

construction. In addition the obtained DNA is almost exclusively of prokaryotic origin. As different microorganisms, may have different susceptibilities to cell lysis methods, the sequence present in the isolated DNA libraries is in somehow dependent on the extraction method.

Delmond et al. (2012) have presented a comprehensive analysis of procedures suitable for DNA extraction from soil samples. These authors applied several DNA extraction procedures that were distinguished based on i) soil sampling sites (horizontal and vertical gradients were considered), ii) cell separation strategy, iii) cell lysis stringency. Surprisingly, the lysis stringency had the strongest effect on the DNA extracted from soil. No one protocol could provide accurate determination of species distribution and therefore only a compendium of different DNA extraction protocols could ensure the best species representativeness.

The variability of microbial communities, taxonomic complexity of libraries derived from different sites was influenced more by depth than by horizontal position of sites (Delmond et al., 2012).

Library sequencing methods

The analysis of genetic diversity of soil metagenoms has recently focused on deep sequencing of 16S rRNA amplicon libraries (Fierer et al., 2007). The 16S rRNA gene is between 1,5-1,6 kb long and after transcription is not translated into protein but assumes a particular secondary structure that allows the assembly of a functional ribosome. The 16S rRNA gene is present in multiple copies in bacterial genome and therefore it is easily amplified by PCR. Based on the level of sequence identity the 16S rRNA sequences have been classified in several regions (see figure 2):

- preserved universal regions: essentially conserved sequence in all bacteria;
- half-preserved regions: conserved for individual belonging to the same taxon;
- variable regions conserved sequences within each single species.

The conventional DNA sequencing approach is based on the dideoxinucleotide chemistry introduced by Sanger et al. (1977). This technique is capable of recovering up to 1 kb of DNA sequence per reaction. The most automated Sanger sequencers can analyze up to 96 reactions in a single run providing sequence information for about 100 kb.

In the last few years, a series of high throughput sequencing devices have been developed based on different chemistry and sequencing techniques (Shokralla et al., 2012).

These Next Generation Sequencing (NGS) technologies can potentially generate hundred thousand to ten million of sequences reads in parallel. In addition, these technologies generate sequencing reads from highly fragmented genomic libraries or amplicons library obtained by PCR from genomic or retro-transcribed RNA molecules and for these reasons are widely used for metagenomic analysis. NGS technologies can be classified into two main categories: the first group included PCR based technique such as Roche 454 Genome Sequencers, HiSeq2000 (Illumina Inc., San Diego, CA, USA); AB Solid TM system (Life Technologies Corp., Carlsband, CA, USA); Ion Personal Medicine (Life Technologies, South San Francisco, CA, USA); Heliscope (Helicos Bioscience Corp. Cambridge, MA, USA) and PACBio RS SMART System (Pacific Bioscence, Mulo PArk, CA, USA), that are suitable for single nucleotide sequencing are members of the second group.

To illustrate the potential of each NGS technique we report below a brief description of the most widely used NGS sequencing techniques in metagenomics studies.

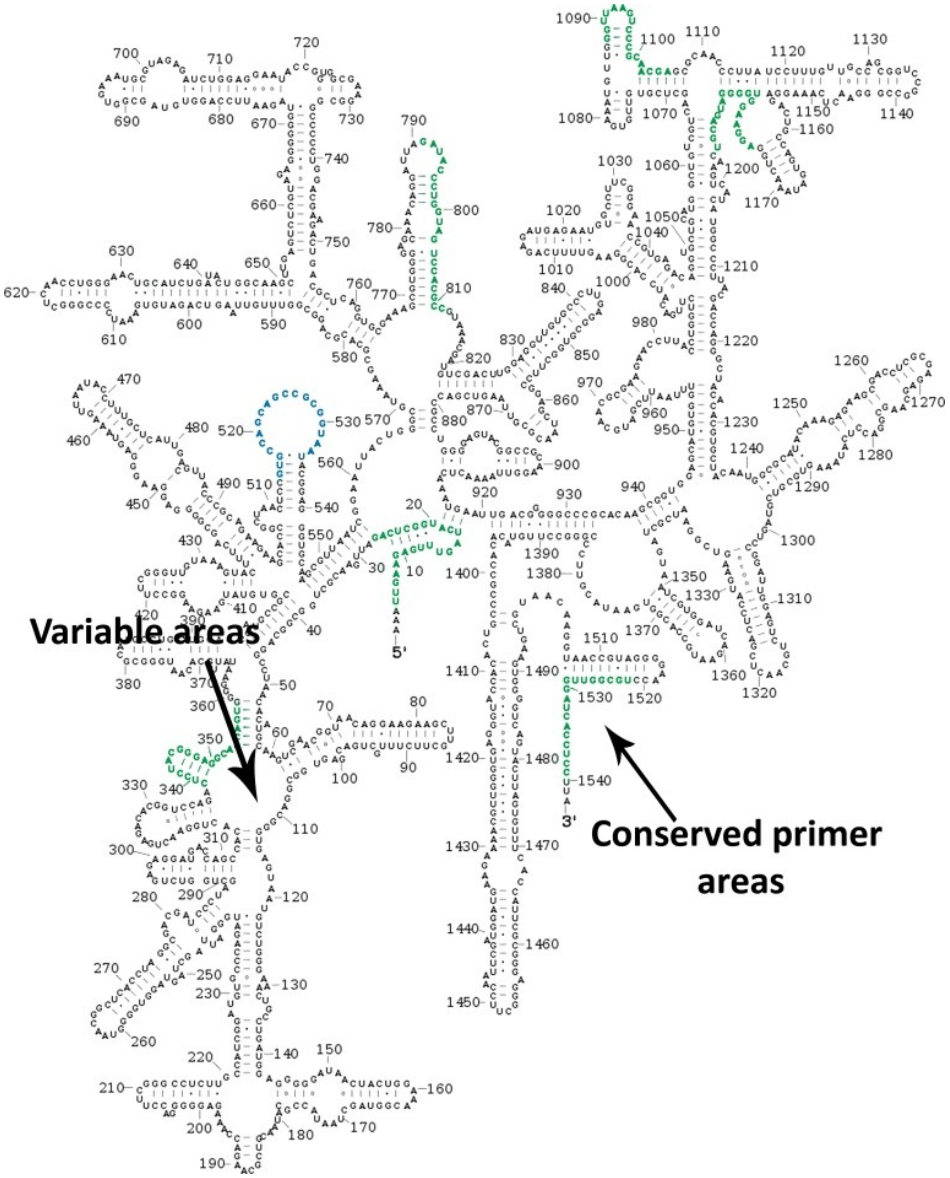

Figure 2: Regions of gene 16S rRNA (image retrieved from www.nature.com)

PCR based next generation sequencing

Roche 454 genome sequences

The Roche 454 sequencing system was developed to exploit the pyrosequencing technology (Margulies et al., 2005). In brief, for each nucleotide incorporated by DNA polymerase a pyrophosphate molecule is released and this promotes a series of downstream reactions that culminate with luciferase mediated light emission. The amount of generated light is directly proportional to the number of nucleotides that are incorporated (see figure 3).

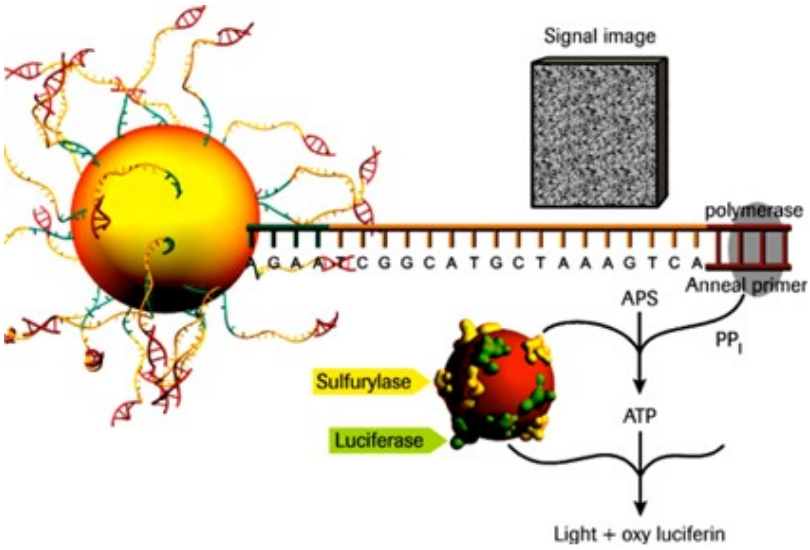

Figure 3: Schematic process of pyrosequencing (image retrieved from Roche, 2005)

The 454 workflow includes:

- PCR amplification of target sequence with primers containing specific adaptors and
- immobilization of the library fragments on either sepharose or styrofoam beads, a step mediated by the sequence complementarity between primers adaptors and the adaptor sequence linked to the beads.

The subsequent step is amplification by the so called "emulsion PCR". The beads are arrayed into Picotiter Plates (PTP) that has millions of wells per plate. Each of the wells can hold one amplicon per DNA beads. Four layers of engineered beads are deposited into the PTP. The PTP is then sequenced en masse in the 454 GD pyrosequencing instrument.

Since each bead is opposite to a CCD camera, light emission following incorporation is digitally registered. The most recent sequencing systems provides 200 nucleotides flow cycles to generate up to 800 bp sequence reads (Shokralla et al., 2012).

Illumina sequences

The Illumina platform utilizes a sequencing by synthesis approach coupled with a bridge amplification on the surface of a flow cell (see figure 4).

Each flow cell is divided into eight separate lanes. Specific adaptor sequences are covalently attached to the interior surface of each cell. These adaptors are complementary to specific adaptors that have been used to generate the DNA library. Hybridization of DNA fragment to oligo (blocked on the flow) occurs by cooling and heating steps. This is

followed by a subsequent incubation with the amplification reactants and amplification to generate millions of clusters.

Each cluster is supplied with polymerase on four differentially labeled fluorescent nucleotides that have their 3'OH chemically inactivated allowing only a single base to be incorporated. After each nucleotide addition, the incorporated nucleotides for each cluster are identified by image recording the excitation step. Finally a deblocking treatment removes the fluorescent group leaving the OH free for the incorporation of the next nucleotide during the next flow cycle step.

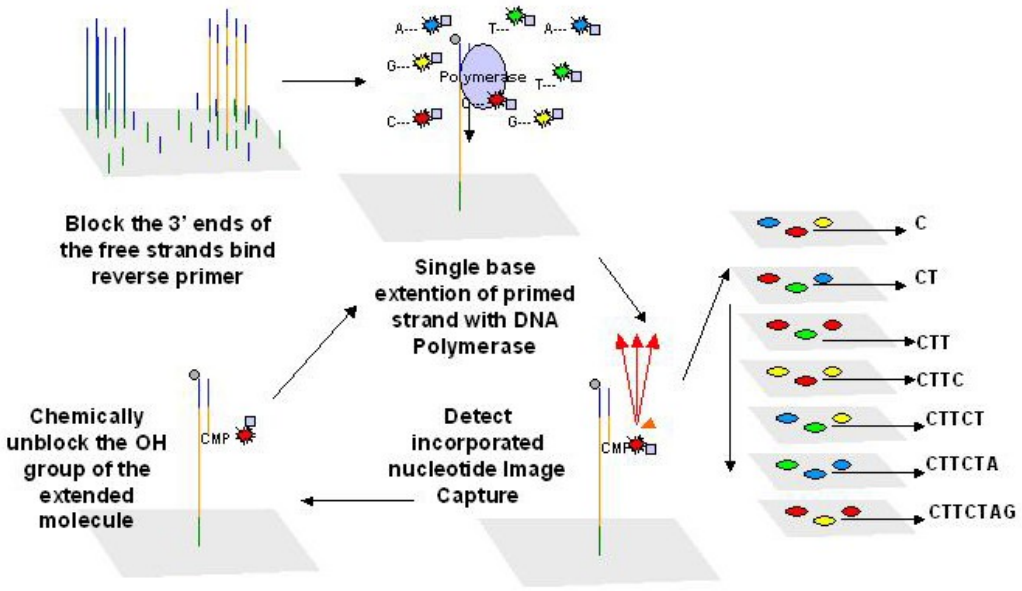

Figure 4: Illumina sequencing method (image retrieved from www.ipc.nexgenomics.org)

Sequencing by oligo ligation

This process couples oligo adapter linked to DNA fragments with complimentary oligo immobilized on the surface of magnetic beads after through emulsion PCR amplification are and the bound to the surface of a special glass (see figure 5). The ligation based process starts with the annealing of a universal sequencing primer that is complementary to a SOLID specific adapter ligated to the library fragment. Four semi-degenerate 8 mer fluorescent oligo are then added along with DNA ligase. When an 8 mer is complementary to DNA fragment sequence, DNA ligase seals the phosphate backbone. Following the ligation step a fluorescent readout identifies the ligated 8 mer oligo, which corresponds to one of the four possible bases.

The 8 mers are cleaved between the fifth and sixth bases to removes the fluorescent group and enabling the next ligation round. The subsequent step is initiated with hybridization of n^{-1} positioned universal primers and subsequent round of oligo ligations. The same process is repeated with n^{-2} and n^{-3} and n^{-4} positioned universal primers.

The generated fluorescence from the five universal primers is decoded with a two base calling software.

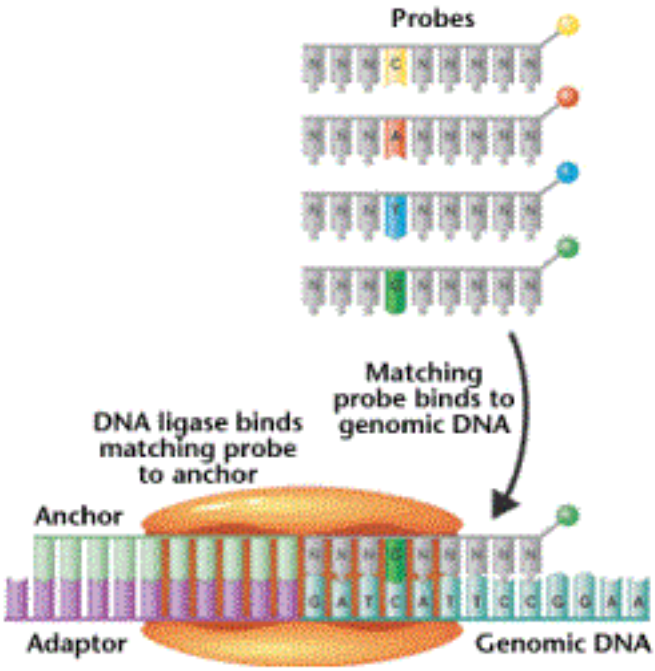

Figure 5: Oligo ligation sequencing method (image retrieved from www.esciencentral.org)

ION torrent

ION torrent relies on the real time detection of hydrogen ion concentration that is released when a nucleotide is added into a DNA strand by PCR (see figure 6). Ion torrent uses high density array of wells wich contain a single DNA template from the library. The wells have a surface composed of a ion sensitive layer and a proprietary ion sensor to detect the change in hydrogen ion concentration following of nucleotides addition.

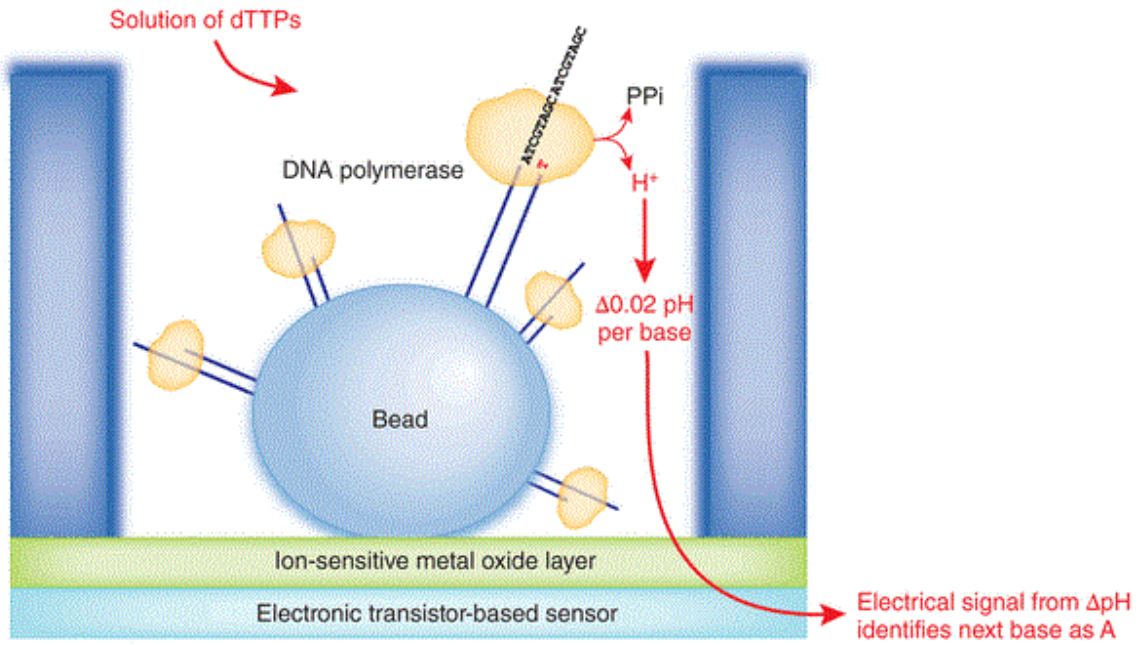

Figure 6: ION torrent sequencing method (image retrieved from www.genomics.cn)

Single molecule DNA sequencing

Heliscope sequencing system is based on the sequencing by synthesis strategy. During the sequencing cycles, the DNA polymerase incorporates a fluorescent nucleotide that is modified to stop the polymerase extension until the incorporated nucleotide's fluorescence is captured.

Following a washing step required to wash off all unincorporated nucleotides fluorescent labels of the incorporated nucleotides are removed by chemical cleavage. Heliscope is capable of producing approximately 1 billion sequence reads.

Pacific Bioscences SMRT DNA sequences

The PAC Bio utilizes a nanostructure, the Zero Made Waveguide (ZMN) for real time observation of DNA synthesis. During the sequencing workflow, the complimentary DNA strand is synthesized from single-stranded template by the action of DNA polymerase. Unlike other technologies, the fluorescent label is attached on the terminal phosphate group rather than nucleotide base, leading to the release of the fluorescence moiety at the time of nucleotide incorporation.

The major advantage of 454 sequencing systems is related to the yield of high number of long reads. In addition, unlike other PCR-based technologies this does not require

de-blocking step to allow DNA extension in the subsequent steps. However, 454 is error prone with template rich in homopolymers with most of sequencing errors being insertion and deletions.

The ILLUMINA, SOLID platforms produce an extremely high yield of short sequence. Indeed read lengths are a major concern for these systems as it can compromise the accuracy of taxonomic assignments.

All sequencing systems based on library PCR amplification may suffer for the bias introduced by the amplification step.

Bioinformatics analysis in metagenomics

Bioinformatic procedures to analyze NGS pyrosequencing data include three core aspects:

- dataset demultiplexing
- reads clustering and taxonomic assignments
- sample comparisons.

Several local or web-based software packages have been developed to trim, filter, compare and visualize amplicon sequence data obtained from NGS. The most widely used are QIIME, Mothur, RDP, VAMPS.

Demultiplexing and quality filter

The initial step with handling raw barcoded sequence data is to de-multiplex the whole sequence set into individual subsets belonging to different samples based on sample specific nucleotide barcodes (see figure 7 (Scholz et al., 2012)).

All reads with a proportion of quality bases or mismatches in the primer or barcodes must be removed before further data processing. QIIME (Caporaso et al., 2010) and mothur have respective command lines script for de-multiplexing and quality filtering. QIIME employs two python scripts or split_libraries.py and split_libraries_fastq.py to perform coupled de-multiplexing and quality filtering and raw data generated by a single 454 runs and Illumina lanes respectively, while mothur depends on Trim sequences to screen and sort pyro-sequences.

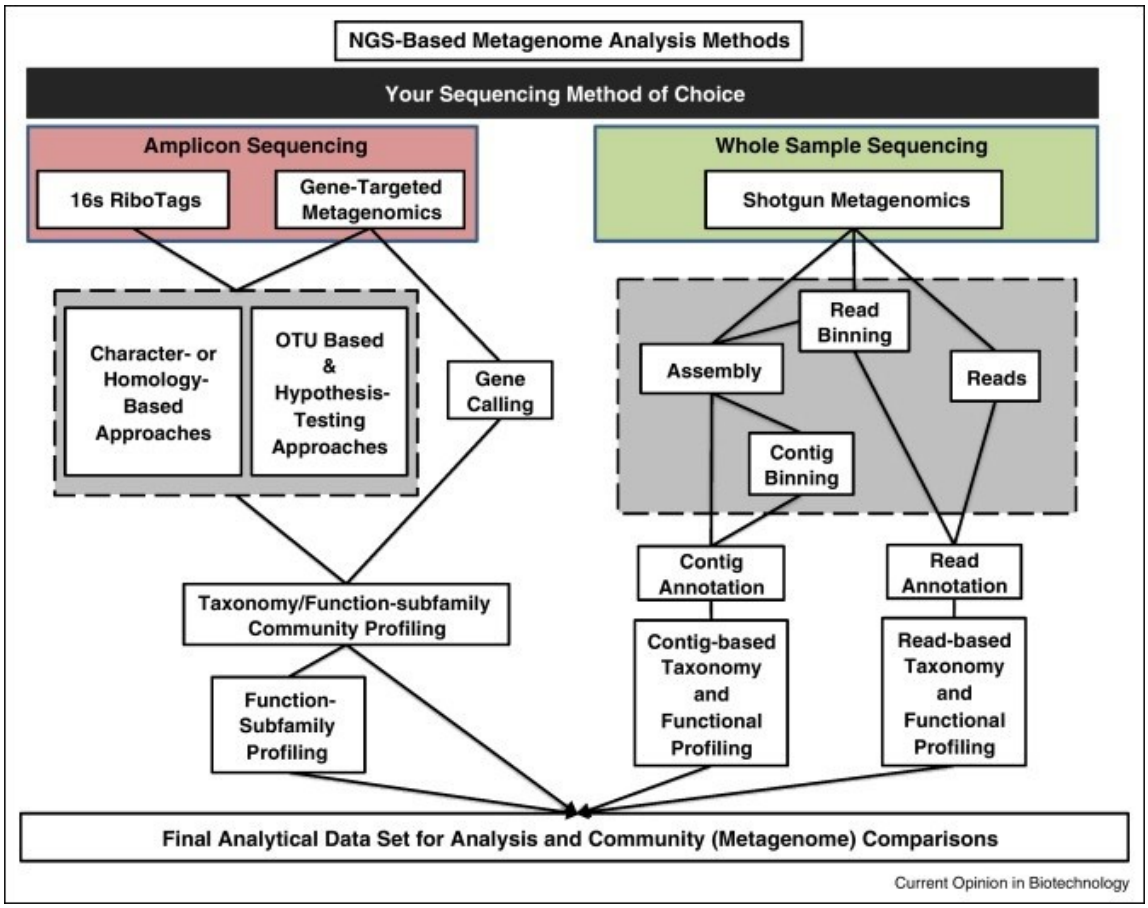

Figure 7: NGS-Based metagenome analysis

As PCR-based amplicons pyrosequencing and other NGS technologies may produce amplification artefacts, a filtering procedure that identifies and eliminates potential artifacts is advisable.

The presence of artifacts may inflate diversity assessments by increasing the number of taxonomically unassigned sequences.

QIIME uses denoiser, other softwares are Amplicon Noise (including PyroNoise and SeqNoise), Acacia (Bragg et al., 2012) and Pro Cluster (mothur) (Schloss et al., 2009). Some of these tools use an expectation-maximization algorithm to identify most likely sequence for every read, while other uses a greedy scheme (Pyronoise). Both these tools are extremely labor intensive and necessitate of high computation capability.

Acacia achieves equivalent sensitivity and specificity for homopolymer error correction from FASTA files, with speeds that are between 200 and 500 fold faster than the Denoiser or Amplicon Noise (Bragg et al., 2012).

Several softwares have been developed for chimera identification such as Chimera Slayer (Schloss et al., 2009), UCHIME (Edgar et al., 2011), Perseus (Quince et al., 2011) and DECIPHER (Wright et al., 2012). However, these software often disagree with one another on the list of identified chimera (Goodrich et al., 2014) probably because conceptual differences in the algorithms.

Data normalization

Data normalization is necessary because samples may obtain different sequencing depth. This outcome could be due to technical (sample independent) or biological (sample dependent) reasons. Two methods, i.e. relative abundance and rarefaction are the most used for pyrosequencing data.

The relative abundance calculated as normalizing sequence counts for a taxon against total sample sequence counts is subjected to statistical pitfalls that can lead to cumulative-based clustering of samples by sequencing depths. The rarefaction method is essentially based on random sampling of an equal number of sequences from each sample. The major drawback with rarefaction method is the loss of valuable sequence data from samples with relative high sequence counts, especially in the presence of large unevenness of sequencing depth across samples, leading to conservative diversity estimates.

OTU picking and taxonomic assignment

After initial quality filtering, denoising and chimera checking, the datasets is analyzed to identify the frequencies of relevant operational taxonomical units (OTUs). OTUs are picked by clustering on the basis of sequence identity. The clustering may occur taking into account the sequence present in the datasets or comparing these sequences against a reference of known OTUs. Various identity cutoffs of 16S gene have been used for different taxonomic ranks. For example identity cutoffs of 99% (for classification at species level) or 97% (classification at family level) or 90% (at order level).

Based on whether to use a reference database, OTU picking strategies are classified in three categories: denovo, closed reference, open reference (Caporaso, 2010). Denovo OTU picking clusters sequences among themselves without a reference database. Closed reference OTU picking matches against a reference database and those unmatched at given identity threshold are discarded.

In open reference OTU picking all sequence are first picked for closed reference OTUs, and any unmatched reads are subsequently clustered for denovo OTUs.

The methods for taxonomic assignment of representative OTU sequence contain three strategies, i.e word matching, best hit and latest common ancestor. The RDP classifier (Cole et al., 2009) is based on word matching and is usually very fast. These powerful tools are used in combination with a reference database (OTUs sets of public well characterized 16S RNA sequence).

References

Alexander M., 1981. Why microbial predators and parasites do not eliminate their prey and hosts. Annual Review of Microbiology, 35:113-33.

Andreson G.L., Caldwell K.N., Beuchat L.R., Williams P., 2003. Interaction of a free-living soil nematode, *Caenorhabditis elegans*, with surrogates of foodborne pathogenic bacteria. Journal of food protection, 66(9): 1543-9.

Bardgett R.D., Jones A.C., Jones D.L., Kemmitt S.J., Cook R., Hobbs P.J., 2001. Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. Soil Biology & Biochemistry, 33: 12-13.

Bragg L., Stone G., Imelfort M., Hugenholtz P., Tyson G.W., 2012. Fast, accurate error-correction of amplicon pyrosequences using Acacia. Nature methods, 9.5: 425-26.

Broughton L.C., Gross K.L., 2002. Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. Oecologia, 125: 420-427.

Caporaso J.G, Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello F.K., Fierer N., Pena A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley J.K., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R.,. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature methods, 7.5: 335-36.

Chodak M., Niklińska M., 2010. Effect of texeture and tree species on microbial properties of mine soils. Applied Soil Ecology, 46:268-275.

Cole J.R., 2003. The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucleic Acids Research, 31.1: 442-43.

Daniel R., 2005. The metagenomics of soil. Nature reviews Microbiology, 3.6: 470-78.

Darbyshire J.F., 1976. Effects of water suctions on the growth in soil of the ciliate colpoda steinii and the bacterium azobacter chroccum. Journal of Soil Science, 27-.369-376.

Delmont T.O., Cecillon R.P., Clark I.M., Constancias F., Simonet P., Hirsch P.R., Vogel T.M., 2011. Accessing the soil metagenome for studies of microbial diversity. Applied and environmental microbiology, 77.4: 1315-24.

Edgar R.C., Haas B.J., Clemente J.C., Quince C., Knight R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics (Oxford, England), 27.16: 2194-200.

Ehrlich H.L., 2002b. Interactions between microorganism and minerals under anaerobic conditions. Geomicrobiology, 459-494.

Felske A., Wolterink A., Van Lis R., Akkermans A. D. L. 1998. Phylogeny of the Main Bacterial 16S rRNA Sequences in Drentse A Grassland Soils (The Netherlands). Applied and environmental microbiology, 64(3): 871-879.

Fierer N., Breitbart M., Nulton J., Salomon P., Lozupone C., Jones R., Robeson M., Edwards R.A., Felts B., Rayhawk S., Knight R., Rohwer F., Jackson R.B., 2007. Metagenomic and Small-Subunit rRNA Analyses Reveal the Genetic Diversity of Bacteria, Archaea, Fungi, and Viruses in Soil. Applied and environmental microbiology, 73.21: 7059-66.

Floate M.J.S., 1970. Decomposition of organic materials from hill soils and pastures. Soil Biology and Biochemistry, 2.3: 173-85.

Floate M.J.S., 1970. Mineralization of nitrogen and phosphorus from organic materials of plant and animal origin and its significance in the nutrient cycle in grazed upland and hill soils. Grass and forage science, 25.4: 295-302.

Forstner U., 1995. Land contamination by metals: global scope and magnitude of problem. In: Allen H.E., Huang C.P., Bailey G.W., Bowers A.R., editors. Metal speciation and contamination of soil. CRC Press; Boca Raton, FL pp. 1–33.

Fuerst J.A., 2005. Intracellular compartmentation in Planctomycetes. Annual Review Microbiology, 59: 299-328.

Garbeva P., van Veen J.A., van Elsas J.D., 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annual Review of Phytopathology, 42: 243–70.

Garrity G.M., Bell J.A., Lilburn T.G., 2004. Taxonomic outline of the prokaryotes release. S.O. Springer, N.Y.

Giardini L., Agronomia generale, 3ª ed., Bologna, Pàtron, 2004.

Goodrich J.K., Waters J.L., Poole A.C., Sutter J.L., Koren O., Blekhman R., Beaumont M., Van Treurer W., Knight R., Bell J.T., Spector T.D., Clark A.G., Ley R.E., 2014. Human genetics shape the Gut Microbiome. Cell, 159.4: 789-99.

Grant S.A., Bolton G.R., Torvell L., 1985. The response of blande bog vegetation to controller grazing by hill sheep. Journal of Applied Ecology, 22: 739-751.

Hattori T., and Hattori R., 1976. The physical environment in soil microbiology: An attempt to extend principles of microbiology to soil microorganism. CRC Critical Review Microbiology, pp. 423-461.

Holben W.E., Jansson J.K., Chelm B.K., Tiedje J.M., 1988. DNA Probe Method for the Detection of Specific Microorganisms in the Soil Bacterial Community. Applied and Environmental Microbiology, 54.3: 703-11.

Huerta-Diaz M.D., Morse J.W., 1992. Pyritization of trace metals in anoxic marine sediments. Geochimica et Cosmochimica Acta, 56:2681–2702.

Janssen P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and environmental microbiology, 72.3: 1719-28.

Kuramae E.E., Yergeau E., Wong L.C., Wong L.C., Pijl A.S., van Veen J.A., Kowalchuk G.A., 2012. Soil characteristics more strongly influence soil bacterial communities than land-use type. FEMS microbiology ecology, 79.1: 12-24.

Kuramae E.E., Gamper H.A., Yergeau E., Piceno Y.M., Brodie E.L., DeSantis T.D., Andersen G.L., van Veen J.A., Kowalchuk G.A., 2010. Microbial secondary succession in a chronosequence of chalk grasslands. ISME Journal, 4 (5): 711-5.

Lauber C.L., Hamady M., Knight R., Fierer N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and environmental microbiology, 75.15: 5111-20.

Lambers H., 1987. Growth, respiration, exudation and symbiontic associations: the fate of carbon translocated to the roots. P.J Gregory, J.V Lake, D.A Rose (Eds.), Root Developmente and Function, Cambridge University Press, pp. 125-145.

Lambers H., 1998. Plant Phisiological Ecology, Springer, New York.

Lorenz P., and Schleper C., 2002. Metagenome-a challenging source of enzyme discovery. Journal of Molecular Catalysis B: Enzymatic, 19-20: 13-19.

Maracchi G., Sirotenko O., Bindi M., 2005. Impacts of present and future climate variability on agriculture and forestry in the temperate regions: European Climate Change, 70: 117-135.

Margulies Marcel., Egholm M., Altman W.E., Attiya S., Bader J.S., Bemben L.A., Berka J., Braverman M.S., Chen Y.J., Chen Z., Dewell S.B., Du L., Fierro J.M., Gomes X.V., Godwin B.C., He W., Helgesen S., Ho C.H., Irzyk G.P., Jando S.C., Alequer M.L.I.,

Jarvie T.P., Jirage K.B., Kim J.B., Knight J.R., Lanza J.R., Laemon J.H., Lefkowitz S.M., Lei M., Li J., Lohman K.L., Lu H., Makhijani V.B., McDade K.E., McKenna M.P., Myers E.W., Nickerson E., Nobile J.R., Plant R., Puc B.P., Ronan M.T., Roth G.T., Sarkis G.J., Simons J.F., Simpson J.W., Srinivasan M., Tartaro K.R., Tomasz A., Vogt K.A., Volkmen G.A., Wang S.H., Wang Y., Weiner M.P., Yu P., Begley R.F., Rothberg J.M., 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature, 437.7057 : 376-80.

McGrath S.P., Zhao F.J., Lombi E., 2001. Plant and rhizosphere processes involved in phytoremediation of metal–contaminated soils. Plant and Soil, 232 (1–2): 207–214.

Nacke H., Thürmer A., Wollherr A., Will C., Hodac L., Herold N., Schöning I., Schrumpf M., Daniel R., 2011. Pyrosequencing-Based Assessment of Bacterial Community Structure Along Different Management Types in German Forest and Grassland Soils. PLOS ONE, 6.2: e17000.

Naether A., Foesel B.U., Naegele V., Wüst P.K., Weinert J., Bonkowski M., Alt F., Oelmann Y., Polle A.,Lohaus G., Gockel S.,Hemp A., Kalko E.K., Linsenmair K.E., Pfeiffer S., Renner S., Schöning I., Weisser W.W., Wells K., Fischer M., Overmann J., Friederich M.W., 2012. Environmental factors affect Acidobacterial communities below the subgroup level in grassland and forest soils. Applied and environmental microbiology, 78.20: 7398-406.

Nannipieri P., Ascher J., Ceccherini M.T., Landi L., Pietramellara G., Renella G., 2003. Microbial diversity and soil functions. European Journal of Soil Science, 54 (4): 655–670.

Nicholson I.A., Patterson I.S., Currie A., 1970.A study of vegetation dynamics: selection by sheep and cattle in Nardes Pasture. Animal Populations in relation to their food resource (ed A. Watson), pp. 129-143.

Ogram A., Sayler G.S., Barkay T., 1987 The extraction and purification of microbial DNA from sediments. Journal of Microbiological Methods, 7.2-3: 57-66.

Oliveira A., Pampulha M.E., 2006. Effects of long-term heavy metal contamination on soil microbial characteristics. Journal of Bioscience and Bioengineering, 102: 157-161.

Pepper I L., Gerba C.P., Newby D.T., Rice C. W., 2009. Soil: A public Health Threat or Savior Critical Reviews in Environmental Science and Technology, 39 (5): 416–432.

Postma J., Walter S., van Veen J:A., 1989. Influence of different initial moisture contents on the distribution and population dynamics of introduced Rhizobium leguminosa rum biovar trifolii. Soil Biology & Biochemistry, 21:437-442.

Quince C., Lanzen A., Danvenport R.J., Turnbaugh P.J., 2011. Removing noise from pyrosequenced amplicons. BMC bioinformatics, 12.1: 38.

Raubuch M., Beese F., 1995. Patterns of microbial indicators in forest soils along a European transect.Biology and Fertility of Soils, 19: 362-368.

Roselló-Mora., and Amann, R., 2001. The species concept for prokaryotes. FEMS Microbiology Reviews, 25:39-67.

Ruess R.W., McNaughton S.J., 1987. Grazing and the dynamics and Energy regulated microbial processes in the Serengeti grasslands. Oikos, 49: 101-110.

Sandaa R..A., Torsvik V., Enger Ø., 2001. Influence of long–term heavy–metal contamination on microbial communities in soil. Soil Biology & Biochemistry, 33 (3): 287–295.

Sanger, F., Nicklen S., and Coulson A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences, 74.12: 5463-67.

Sangwan P., Kovac S., Davis K.E.,Sait M., Janssen P.H., 2005. Detection and cultivation of soil verrucomicrobia. Applied and environmental microbiology, 71.12: 8402-10.

Schimel J.P., Balser T.C., Wallenstein M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology, 88:1386-94.

Schloss P.D., Westcott S.L., Ryabin T., Hall J.R., Hartmann M., Hollister E.B., Lesniewski R.A., Oakley B.B., Parks D.H., Robinson C.J., Sahl J.W., Stres B., Thallinger G.G., Van Horn D.J., Weber C.F., 2009. Introducing mother: Open-source, platform independent, community-supported software for describing and comparing microbial communities. Applied and environmental microbiology, 75, 7537-7541.

Scholz M.B., Lo C.C., Chain P.S.G., 2012. Next generation sequencing and bioinformatic bottlenecks: the current state of metagenomic data analysis. Current Opinion in Biotechnology, 23.1: 9-15.

Shange R.S., Ankumah R.O., Ibekwe A.M., Zabawa R., Dowd S.E., 2012. Distinct soil bacterial communities revealed under a diversely managed agroecosystem. PLOS ONE, 7.7: e40338.

Shi W., Bischoff M., Turco R., Konopka A., 2002. Long–term effects of chromium and lead upon the activity of soil microbial communities. Applied Soil Ecology, 21 (2): 169–177.

Shokralla S., Spall J.L., Gibson J.F., Hajibabaei M., 2012. Next-generation sequencing technologies for environmental DNA research. Molecular ecology, 21.8: 1794-805.

Sobolev D., and Begonia M.F., 2008. Effects of heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. International Journal Environmental Research Public Health, 5(5): 450-6.

Swift M.J., Heal O.W., Anderson J.M., 1979. Decomposition in Terrestrial Ecosystems. Blackwell University of California Press, Berkeley, CA.

Teixeira L.C.R..S., Peixoto R.S., Cury J.C., Jun Sul W., Pellizzari V.H., Tiedje J., Rosado A.S., 2010. Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. The ISME journal 4.8: 989-1001.

Torsvik V., Sorheim R., Goksoyr J., 1996. Total bacterial diversity in soil and sediment communities - a review. Journal of Industrial Microbiology and biotechnology., 17, 170–178.

Wang Q., Garrity G.M., Tiedje J.M., Cole J.R., 2007. Naive classificatore bayesiano per la rapida assegnazione delle sequenze di rRNA nella nuova tassonomia batterica. Applied and environmental microbiology, 73: doi: 10.1128/aem.00062-07.

Wardle D.A., Lavelle P., 1997. Linkages between soil biota, plant litter quality and decomposition. In: Gadish G., Giller K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford, UK, 107–124.

Webster G., Embley T.M., Prosser J.I., 2002. Grassland Management Regimens Reduce Small-Scale Heterogeneity and Species Diversity of a Proteobacterial Ammonia Oxidizer Populations. Applied and environmental microbiology, 68.1: 20-30.

Winding A., Hund–Rinke K., Rutgers M., 2005. The use of microorganisms in ecological soil classification and assessment concepts. Ecotoxicology and Environmental Safety, 62 (2): 230–248.

Wright E.S., Yilmaz s.L., Noguera D.R., 2012. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Applied and environmental microbiology, 78.3: 717-25.

Yap, W. H., Li X., Soong T.W., Davies J.E., 1996.Genetic diversity of soil microorganisms assessed by analysis ofhsp70 (dnaK) sequences. Journal of Industrial Microbiology & Biotechnology, 17.3-4: 179-84.

Zhang H., Sekiguchi Y., Hanada S., Hugenholtz P., Kim H., Kamagata Y., Nakamura K., 2003. *Gemmatimonas aurantiaca* gen. Nov., sp. Nov., a Gram-negative, aerobic, polyphosphate-accumulating micro-organism, the first cultured representative of the new bacterial phylum *Gemmatimonadetes* phyl. Nov. International Journal of Systematic Evolutionary Microbiology, 53, 1155-1163.

CHAPTER 1

Analysis of bacterial community structures along three different grazing managements in Sardinian soils

Abstract

While many studies have analyzed the effect of grazing on aboveground biodiversity, little information is available on soil microbial diversity. In the present work we applied pyrosequencing of 16S rRNA libraries to analyze richness and diversity of bacterial communities of Sardinian soils managed with different grazing intensity. Our data indicated that grazing is associated to a dramatic shift of community structure with *Firmicutes* being positively associated to grazing intensity and becoming the dominant phylum in intensively grazed soils. No significant shifts were noted in the relative abundance at class, order or family taxonomic levels suggesting no drastic changes within phyla. Correlation analysis of bacterial communities composition and physical-chemical properties of soils highlighted significant associations between soil pH or C/N ratio and *Firmicutes* and *Verrucomicrobia* relative abundance.

Introduction

Agricultural intensification is believed as a crucial factor promoting biodiversity losses at several ecological levels (Janssen et al 2006; Myrold et al, 2013). While declines in taxonomic richness of aboveground multicellular communities (plants and animals) have been documented in several agro-ecosystems (Collins et al., 1998; Lawton et al., 1998) little information is available on below-ground diversity (Bardgett et al., 2001). Recent studies of metagenomics have shown that both soil characteristics and land management influence soil microbial diversity. Souza et al. (2013) analyzed the effects of conventional tillage with crop rotation and succession with major differences being attributed to tillage and to a lesser extent to crop management. Acosta-Martinez et al. (2008), have shown that tillage reduces bacterial diversity due to the alteration of physical structures of soil. Through tillage, organic matter incorporated trough the plough layer of the soil would benefit unique microbial communities causing them to revert to an early and more unstable stage of "ecological succession". These communities are proposed as having quick response to condition of "feast and famine" that will be generated by growing season-fallow in cultivated systems. Torsvick et al. (1998) analyzed the effect of human induced pollution on soil biodiversity and concluded that soil management can dramatically compromise bacterial diversity. Other studies have analyzed the effect of introducing organic amendments to soil concluding that biochemical changes of soil are often accompanied by significant changes in microbial diversity. Shange et al (2012) have analyzed replicates soil samples collected from three land use systems (grazed pine forest, cultivated crop, and grazed pasture) all deriving from a single soil type. A reproducible trend of microbial diversity reduction was observed passing from grazed pasture to cultivation and forested pines.

The increased richness of grazed samples was attributed to three major factors: i) organism and substrate diversification from fecal and urine deposition; ii) stimulation of rhizosphere activity as a consequence of mowing and the iii) mixing and dispersal of microbial communities through trampling (Parham et al., 2005; Enwall et al., 2007). The low diversity of forested pines was explained by the harsh conditions to resident organisms, specifically in the acidic pH and polyphenolic compound found in loblolly pine litter (Naether et al., 2012). Specific phylum such as *Actinobacteria*, *Acidobacteria* and *Proteobacteria* showed significant shifts across the land use strata. *Actinomycetales*

and *Solirubrobacterales* orders showed their highest abundance in the heavily disturbed cultivated systems. Selected soil properties also differed across different land use regimes with pH showing variations consistent with shifts of communities structure and composition. Kuramae et al. (2011) have suggested that the effects due to land use managements although significant are less important than those associated to soil characteristics.

Bardgett et al. (2001) have analyzed soil microbial communities across a successional transition in submontane regions of UK. These authors found that on heavily grazed soil the bacteria flora has a greater role than fungi while the opposite behavior was found for nearly unmanaged and ungrazed soils. Moreover, the bacterial diversity was negatively associated with the grazing intensity indicating a trend toward specialization with increasing stress conditions. However the higher level of bacterial diversity and phenotypic evenness were observed in soils with low to medium grazing intensity, a finding that lend support to the 'grazing optimization hypothesis' that suggest that ecosystem productivity, especially primary productivity reaches a maximum at moderate levels of herbivory (Hilbert et al., 1981). A such effect indicates that decomposer related processes, such as nutrient cycling, might also be optimum at light to intermediate grazing levels. In the present work we analyzed the level of bacterial diversity of Mediterraneans soils subjected to different grazing intensive by sheeps. Our experimental setup consisted of soil samples taken from sites that were subjected to different grazing intensitiy. The proximity of sampling sites ensued a fairly common geological origins of sites while historical records of land use management accounted for the grazing intensities difference across samples.

Materials and methods

Study area

The soil samples were collected during November 2011. The collection sites were located in the area of Berchidda-Monti Long Observatory (Olbia- Tempio, 40°47’6”36 N, 9°9’55”,80 E) (see figure 1). This area is considered meso-Mediteranean, subhumid phytoclimatic belt with annual rainfall averages of 862 mm or mean temperature of 13,8°C. The samples represented three distinct soil management conditions.

- Intensive Grazing (IG): grassland managed according to a flexible rotational scheme consisting of a fallow pasture which is cropped every from 2 to 5 years depending on the thorny vegetation and other low pastoral value species presence. The annual hay crop includes Italian ryegrass (*Lolium multiflorum*) and annual clovers, among which Italian clover (*Trifolium incarnatum*), subclover (*Trifolium subterraneum*) and balasae clover (*Trifolium michelianium*). Grazing pressure was about 0,8-1 livestock units (LSU) ha^{-1} during the whole year.
- Wooded Pasture (WP) an area with 15 to 35 cork-oak trees per ha^{-1}. Grazing pressure as for IG.
- Wooded Land (WL) Grazing pressure ranging from 0,2 to 0,3 livestock units over a period of 30 days per year. The tree density is 15-35 cork-oaks ha^{-1}.For this treatment soil samples were taken under the tree (WPU) and at least 5 meter apart from the closest tree (WPO).

Nucleic acid extraction and amplification

Total genomic DNA extraction from soils was performed with the PowerSoil™ DNA Isolation Kit Sample (Mobio Laboratories, inc., corporate headquarters 2746 Loker Avenue West) according to manufacturer's instructions.

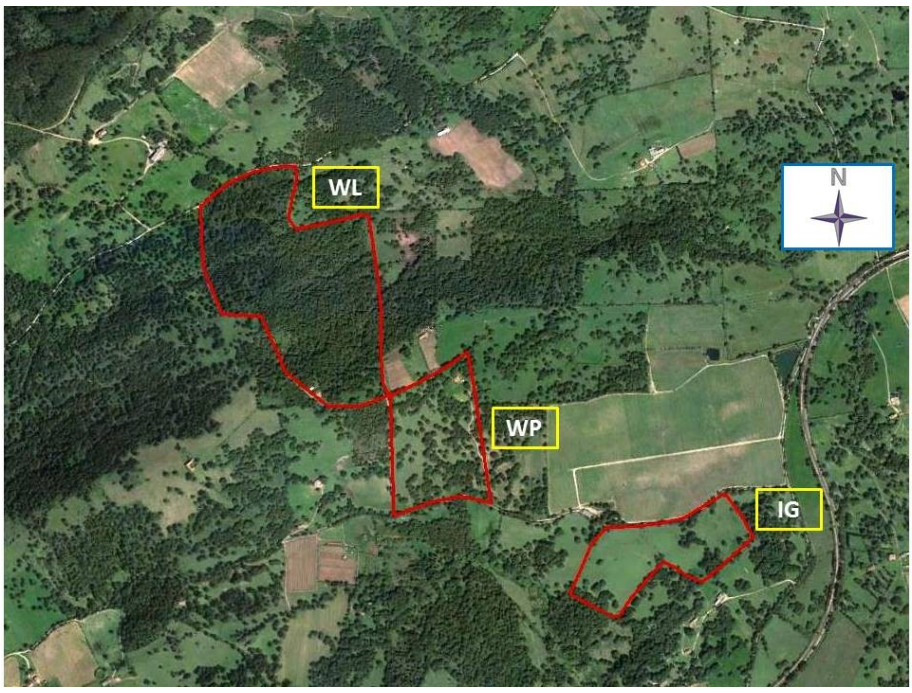

Figure 1: Aereal photo of ampling sites in Berchidda

PCR reaction was composed of 10 mM for each dNTPs; Buffer 10x; 50 mM $MgCl_2$; 10 µM for each primers in a total volume of 50 µl.

The reverse primer (2 µl for each sample) has the following sequence CGTATCGCCTCCCTCGCGCCATCAGGGATTAGATACCCBRGTAGTC.

PCR amplification was carried out on genomic DNA with 16S rDNA specific primer with 454 adaptors that have at unique MIDs sequences (see table 1).

The program for the amplification by PCR includes an initial denaturation at 94 °C for 3', 35 cycles of denaturation at 94 °C for 30", annealing at 55 °C for 30" following by extension at 72 °C for 40". All amplicons were cleared using ampure DNA capture beads and pooled in equimolar concentrations.

Sample ID	Barcode Sequence	Linker Primer
IG1	ATACGACGTA	TCACGRCACGA
IG2	CATAGTAGTG	TCACGRCACGA
WLO1	AGACGCACTC	TCACGRCACGA
WLO2	ACGCTCGACA	TCACGRCACGA
WLU1	ACGAGTGCGT	TCACGRCACGA
WLU2	TCTCTATGCG	TCACGRCACGA
WPO1	ACGAGTGCGT	TCACGRCACGA
WPO2	CGTGTCTCTA	TCACGRCACGA
WPU1	CGTCTAGTAC	TCACGRCACGA
WPU2	ATATCGCGAG	TCACGRCACGA

Table 1: List of the sequence of the primers

Pyrosequencing and statistical analysis

Sequencing of 16S rRNA amplicons was performed at the Macrogen sequencing service (Macrogen Korea 10F, 254 Beotkkot-ro Geumcheon-gu, Seoul) with a Roche GS-FLX 454 Pyrosequencer. Sequences that were shorter than 200 bp in length and with a quality score below 25 were discarded. The OTUs were identified using a clustering approach as implemented in the software uclust and with a similarity threshold of either 3% or 20% (Edgar, 2010). For taxonomy based analysis the RDP classifier of the ribosome database project (RDP) was used at a confidence level of 80% (Wang et al., 2007). The alpha diversity indexes were calculated on randomly picked datasets containing a predefined number of sequences. For each of these randomly generated

datasets we studied the total number of observed OTUs (operational taxonomical units) and the non parametric chao1 index. Rarefaction curves were constructed for the alpha diversity indexes on datasets with an increasing number of sequences (from 1 to 40000).

Principal component analysis was performed with the JMP software (SAS Institute Inc., Cary, NC, 1989-2007).

ANOSIM was used to calculate non parametric analysis of variance (Caporaso et al., 2010). Pairwyse correlation analysis between relative abundance of taxonomic classes and phisico-chemical of soil were performed with the JMP 7,0 suite. Canonical correspondence analysis was performed with the PAST software (Quast et al., 2013).

The alpha diversityes values were composed using a non parametric t-test which used Monte Carlo permutations to determine the p-value (Caporaso et al., 2010).

Results and discussion

Phisico-chemical properties of soils

In the present study we analyzed the composition of bacterial communities present in soils sampled. The samples represented replicates of three management systems differentiated for the grazing pressure and presence of trees.

Physical and chemical characteristics of sampled soils are reported in table 2. The grazing pressure was estimated in the order of 0,8-1 livestock unit for intensive grazed samples (for the whole year; IG) and wooded pasture samples WP. The livestock unit of wooded land (WL) was 0,2-0,3 livestock unit (for about 30 days/year).

Bacterial richness and diversity

A total of 445789 sequences were obtained by pyrosequencing of amplicons obtained with 16S V2-V3 specific primers on total DNA extracted from five soil samples. The number of sequences was reduced to 315091 after filtering sequences shorter than 200 bp and/or showing an average quality score below 25. WPU2 (36119) and WLO2 (35803) were the treatments with the highest number of sequences while WLU1 showed the lowest number (22240). The average length of filtered reads was 255 bp.

Sequences were assigned to OTUs based on either 97% or 80% similarity. The effect of sampling on richness and diversity was analyzed by rarefaction based approach.

The number of observed OTUs increased with the number of sequences per sample for the dataset obtained with a sequence similarity of 3% and almost none of the curves reached saturation (see figure 2).

Properties	WLO1*	WLO2*	WLU1*	WLU2*	WPO1*	WPO2*	WPU1*	WPU2*	IG1*	IG*2
Fine loam (2/20 μm) g/kg	141	140	132	141	122	110	108	118	123	136
Coarse loam (20/50 μm) g/kg	69	65	66	72	84	71	70	71	72	85
Fine sand (50/200 μm) g/kg	137	140	120	114	152	134	156	144	141	159
Coarse sand (200/2000 μm) g/kg	513	516	536	507	498	545	537	521	520	444
Organic C g/kg	24	27	30	36	22	21	32	27	20	22
Total N g/kg	1,5	1,6	1,7	2,1	1,6	1,5	2,2	1,8	1,6	1,7
C/N	16	17	17	17	14	14	15	15	13	13
Organic matter g/kg	41,2	45,4	52,4	63,0	37,1	36,1	56,1	46,8	36,1	38,1
Ph	6,2	6,1	6,0	5,9	5,8	5,8	5,7	6,0	5,1	5,1
P_2O_5 Olsen g/kg	0,006	0,006	0,007	0,011	0,012	0,012	0,015	0,020	0,04	0,030
Ca cmol+/kg	4,81	5,57	5,34	6,43	5,38	6,42	6,37	7,20	2,57	2,78
Mg cmol+/kg	1,86	1,64	2,13	2,28	1,72	1,89	2,00	2,44	0,64	0,60
Na cmol+/kg	0,206	0,234	0,289	0,230	0,206	0,194	0,208	0,203	0,15	0,136
K cmol+/kg	0,212	0,212	0,333	0,436	0,132	0,199	0,296	0,601	0,37	0,345
Fe mg/kg	0,005	0,009	0,005	0,005	0,012	0,006	0,011	0,011	0,01	0,016
Al cmol+/kg	0,113	0,164	0,155	0,156	0,243	0,203	0,217	0,156	1,011	0,968
Cu mg/kg	0,126	0,122	0,131	0,146	0,104	0,094	0,135	0,115	0,13	0,159
Mn mg/kg	8,82	22,83	35,00	38,00	18,60	15,96	47,80	19,09	22,7	18,00

Table 2: Physical and chemical characteristics of soils (*see table 1 for soil coding system)

At clustering threshold of 80% (see figure 3) most of the rarefaction curves reached saturation, indicating that the surveying effort covered almost the full range of taxonomic diversity detectable at this genetic distance. Alpha diversity analyses were performed on randomly re-sampled dataset, each composed of 20000 sequences.

Considering a surveying effort of 20000 it was estimated that the total diversity recovered in our re-sampled datasets was in the range of 58% to 69%. Thus, we did not analyze the full extent of genetic diversity at these genetic distances but a substantial fraction of bacterial diversity was assessed at species and genus level.

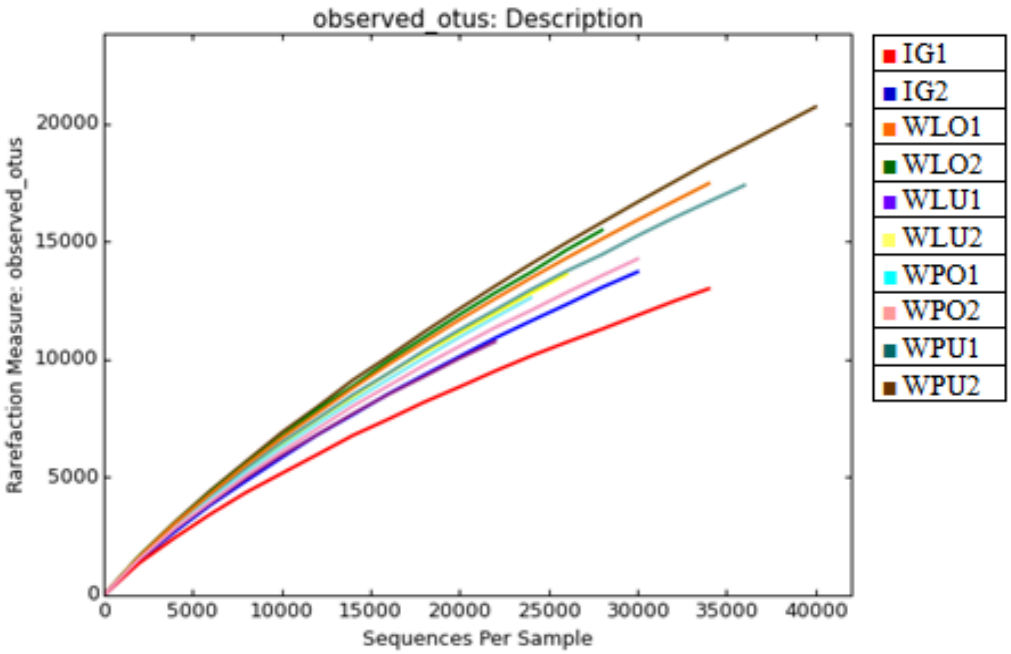

Figure 2: Rarefaction curve (observed_OTUs) at a sequence similarity level of 3%

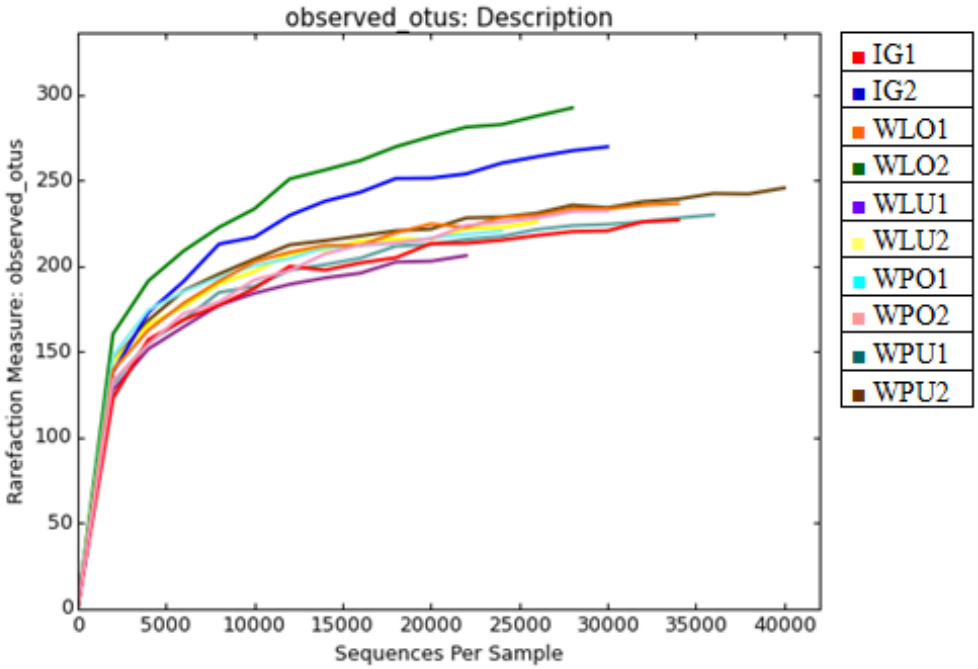

Figure 3: Rarefaction curve (observed_OTUs) at a sequence similarity level of 20%

The bacterial richness estimated by the number of OTUs and, Chao1 non parametric richness estimator are reported in table 3.

	Chao1 97%	OTUs 97%	Chao1 80%	OTUs 80%
IG1	27148,01	8847	246	211
IG2	32751,61	10128	279	249
WLO1	44477,31	11944	324	271
WLO2	40531,24	11629	279	226
WPO1	37072,42	10952	230	217
WPO2	33518,28	10544	244	220
WPU1	42267,67	12102	241	225
WPU2	37179,78	11296	246	219
WLU1	29043,34	10046	216	200
WLU2	36709,64	11150	235	212

Table 3: Bacterial richness as estimated by number of otus and Chao1

Bardgett et al. (2007) have reported a negative association between species richness and grazing intensities in successional transitions of submontane UK soils. We compared the species richness of intensively (WPO) and lightly grazed samples (WLO) taken from wooded sites. For both Chao1 and Observed_OTUs indexes, the higher grazing intensities were associated with lower richness but the difference was not significant (P>0,05). A similar picture was obtained when comparing the IG and WPO samples to the other samples (WPU, WLU and WLO). A positive influence of tree on bacterial richness and diversity was already indicated by Nacke et al. (2011) in a survey of bacterial community samples of German soils with different managements. The presence of trees had a controversial effect on bacterial richness of our samples.

As we could see from the indexes of bacterial diversity at the highest grazing intensities a positive, though not significant effect of tree (P>0,05) on bacterial richness was observed (IG *vs* WPU). However such a effect was not reproduced for the comparison at lower grazing intensities (WLU *vs* WLO), with the Chao1 index being higher in WPU than in WPO sites.

Taxonomic structure of bacterial communities

The assignment of OTUs to bacteria phyla was obtained by the RDP classifier using the GreenGenes version 2,1 as subject database (Wang et al., 2007). The distribution of different phyla in the analyzed samples is reported in figure 4 while the absolute counts are reported in table S1.

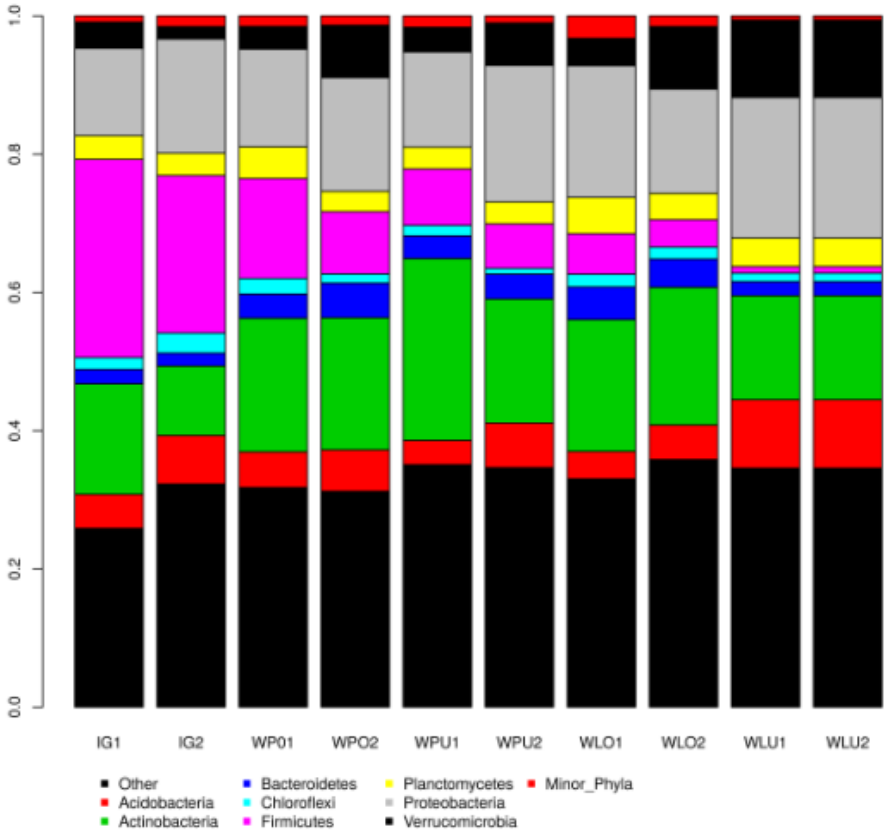

Figure 4: Relative abundance of bacteria phyla across samples

The most represented phyla were *Actinobacteria* (17,8%) followed by *Proteobacteria* (16,5%) and *Firmicutes* (10,3%). On average, one third of OTUs was not assigned to known phyla suggesting an high microbiome peculiarity for the analyzed soils. The distribution of bacteria assemblages in different samples was studied by performing a principal component analysis on phyla abundances (see figure 5).

The first two axes explained 32% and 29% of total variance. The intensively grazed samples (IG1 and IG2) were clearly separated from other samples especially along the second axis. The analysis of loading plot indicated the *Firmicutes* as the major phyla contributing to this difference (the autovector matrix is reported in table S2). The WLU

samples were separated from other wooded samples along the first axis and in this case the loading plot indicated the *Acidobacteria* as the most weighing phylum (see Figure 6).

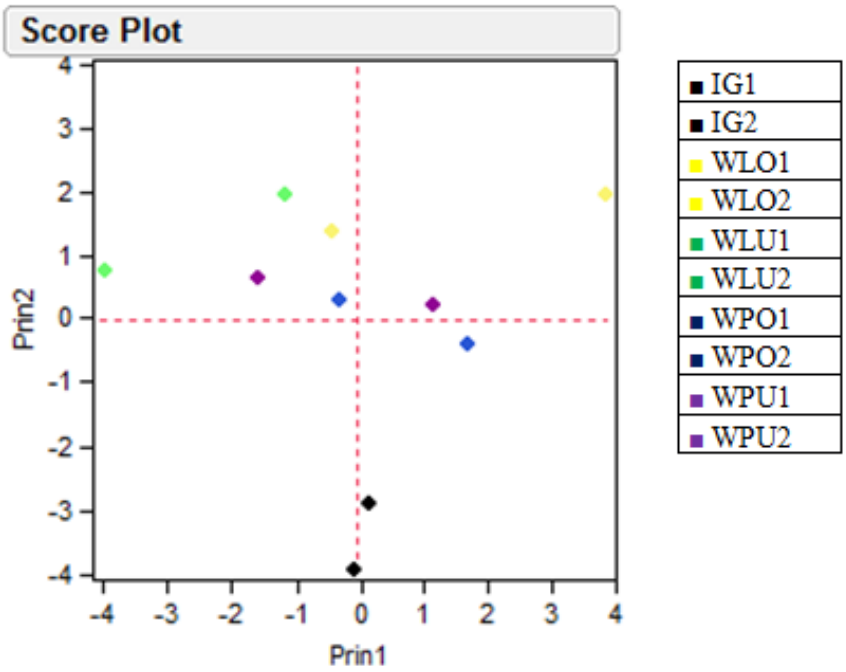

Figure 5: Score plot principal component analysis bacterial

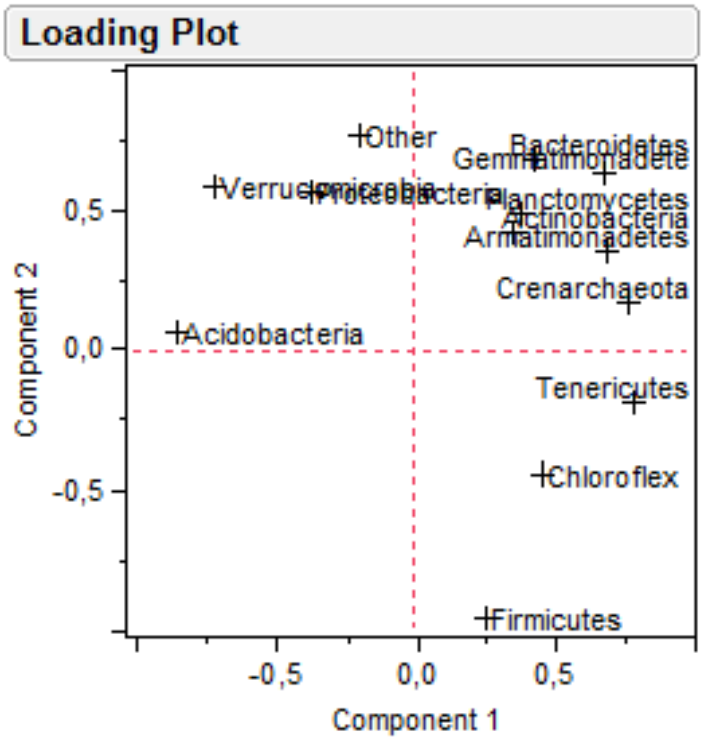

Figure 6: Loading plot of the principal two axes bacterial

Other classes showed low weights on both axes being detected with comparable abundance in all treatments (absolute counts of classes are reported in table S3).

To gain deeper insight on bacterial communities we analyzed relative abundance of bacteria at class and order taxonomic levels. The relative abundance of *Firmicutes* was associated to grazing intensities (see figure 4): the highest relative abundance was observed in intensively grazed samples: 22,7% for IG2 and, 28,6% for IG1 followed by WP samples: 14,4% and 9% for WPO1 and WPO2, respectively. The WP samples with less intensive grazing pressure, WPU showed lower relative *Firmicutes* abundance from 3,4% for WPU1 to 8,1% for WPU2. Finally the relative abundance of *Firmicutes* in WL ranged from a minimum of 2,7% for WLU1 to maximum of 5,8% of WLO2.

As shown in figure 7 most of *Firmicutes* belonged to the class of *Bacilli*. Within this class the majority of OTUs were assigned to a not yet characterized order.

The *Bacillales* was the most abundant class among characterized *Firmicutes* orders (absolute counts of orders are reported in table S4).

Nacke et al. (2011) demonstrated an association between *Firmicutes* enrichment and the prevalent species at grazing (sheep>horse pastures). However, the relative abundance of *Firmicutes* reported in this study was exceptionally high (2-3 folds higher than the level reported in Nacke study). Further analyses are needed to understand whether this observation can be explained by grazing intensity or by other pedological or management conditions.

The *Alphaproteobacteria* and the *Betaproteobacteria* were the most represented classes of *Proteobacteria* (see figure 8).

The proportion of sequences not assigned to a known class was below 15%. No clear distinction were observed across samples for the *Proteobacteria* orders.

The *Acidobacteria* are particularly abundant in acidic soil covered by forest. In our samples the *Acidobacteria* were found at a relative abundance of 17 to 20% (see figure 4). The analyses carried out a class level showed an unusual abundance of *Solibacteres* in IG soils compared to other soils (see figure 9).

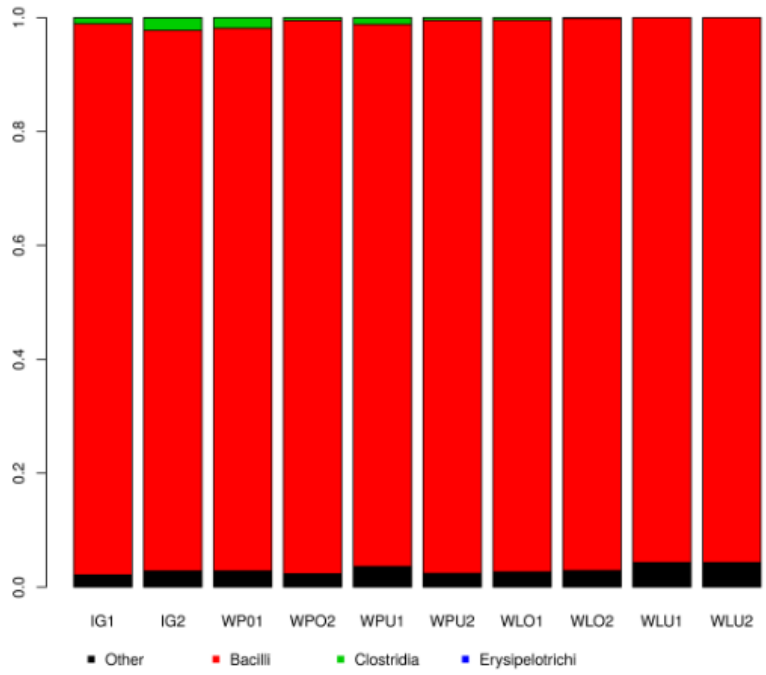

Figure 7: Relative abundance of *Firmicutes* class across samples

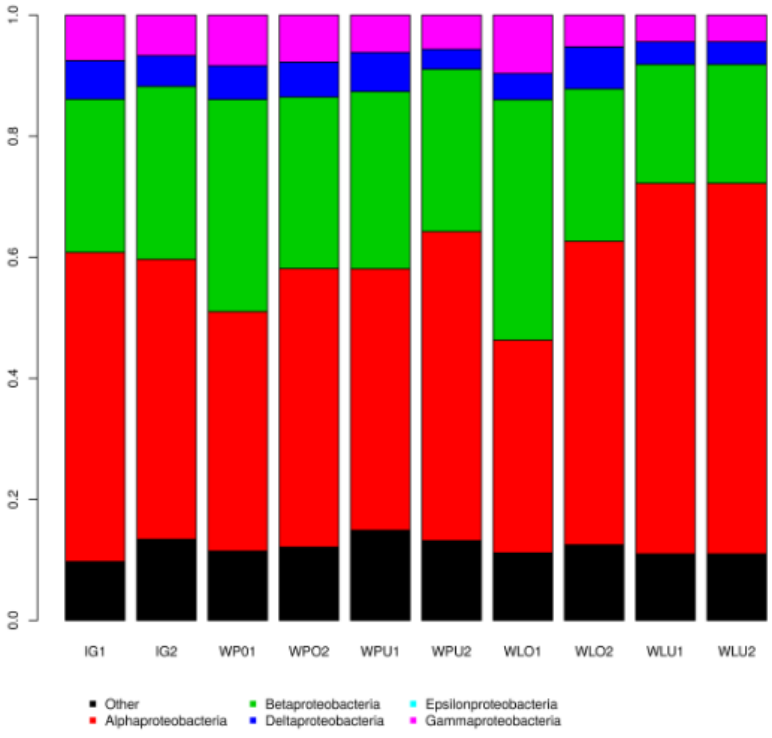

Figura 8: Relative abundance of *Proteobacteria* class across samples

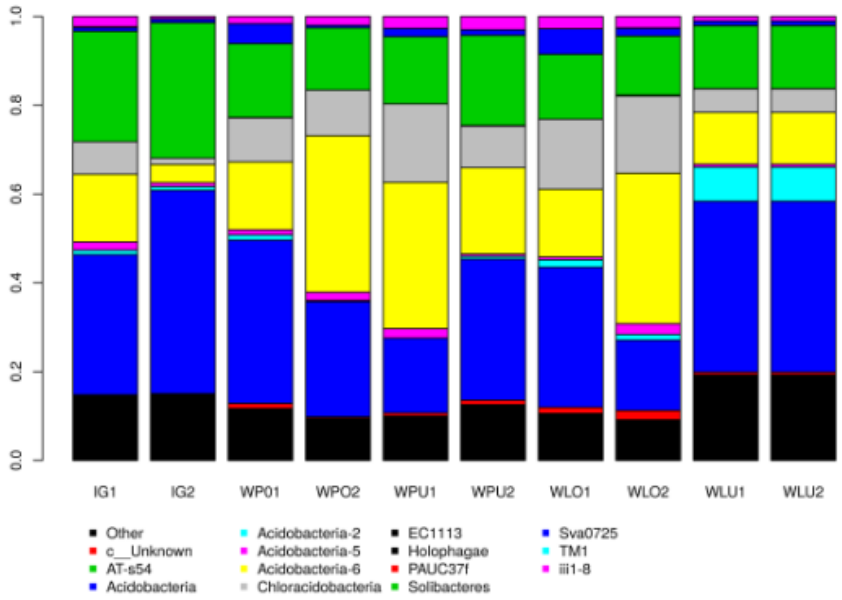

Figure 9: Relative abundance of Acidobacteria class across samples

The *Actinobacteria* were characterized by a low taxonomic depth, with only 5 classes represented in analyzed soils (see figure 10). The *Actinobacteria* was the most represented class (see figure 10) and within this class the *Actinomycetales* was the most abundant order (data not showed). No relevant difference were noticed across samples.

The absolute counts of family bacterial are reported in table S5.

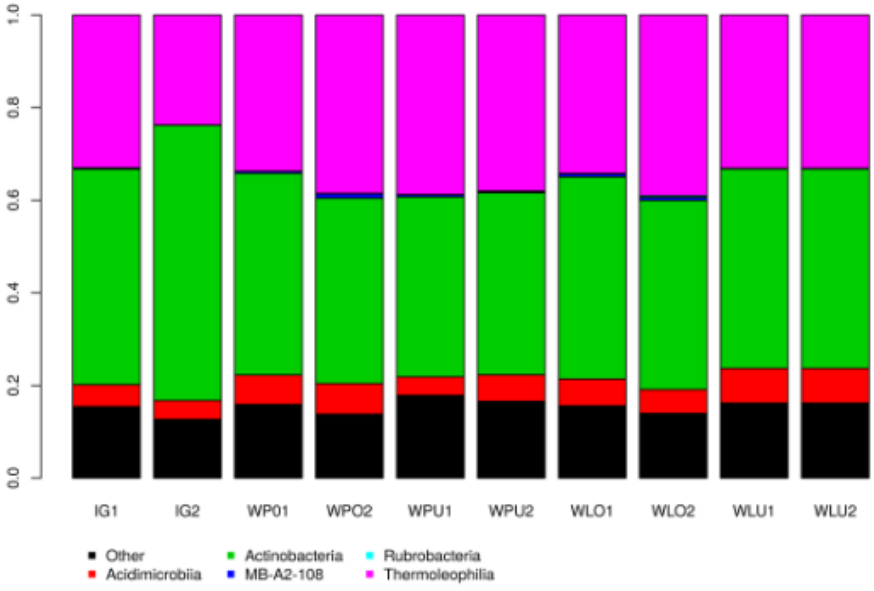

Figura 10: Relative abundance of *Actinobacteria* class across samples

Relation between physico-chemical properties and bacterial community structures of sampling sites

The relations between physico-chemical properties of soil and bacterial community structures were analyzed by a canonical correspondence approach (CCA). The CCA triplot of figure 11 reports the first two CCA axes (the two axes explained 74% and 15% of total inertia), respectively.

We found that *Firmicutes* were associated with low pH and C/N ratio and high P content. These relations were confirmed by linear regression analysis (see figure S1).These results are consistent with previous studies that reported a large proportion of *Firmicutes* in slightly acidic grassland soils. Kuramae et al. (2011) have described bacterial communities with OTUs assigned to Bacilli and Clostridia positively associated to P content. This observation was interpreted as the results of decades of external inorganic and organic fertilizer inputs to these soils (Bruchem et al., 1999). An opposite scenario was found for *Verrucomicrobia* that were found associated with high C/N content and low P (see figure 11)

Conclusions

In the present study we analyzed the microbial community structure of soil samples that were subjected to different land uses. Principal component analysis indicated that sample sites were distinguished along the first two components with intensive grazed samples (IG) being clearly separated by other samples. *Firmicutes* were the bacteria phylum that contributed more significantly to a such separation. Indeed Kuramae et al. (2010) have reported a similar picture for chalk soils that were intensively fertilized.

Under this scenario the *Firmicutes* will be particularity abundant because of the high nutrients inputs. Analysis conducted at other taxonomic levels demonstrated that most *Firmicutes* belonged to the class of *Bacilli* order *Bacillaceae.* Mandic et al. (2015) have recently reviewed the involvement of *Bacillaceae* in the degradation of soil organic matter and plant litter. Most members of the *Bacillaceae* are aerobic heterotrophic saprophytes that are capable of degrading a range of polymeric carbonaceous substances.

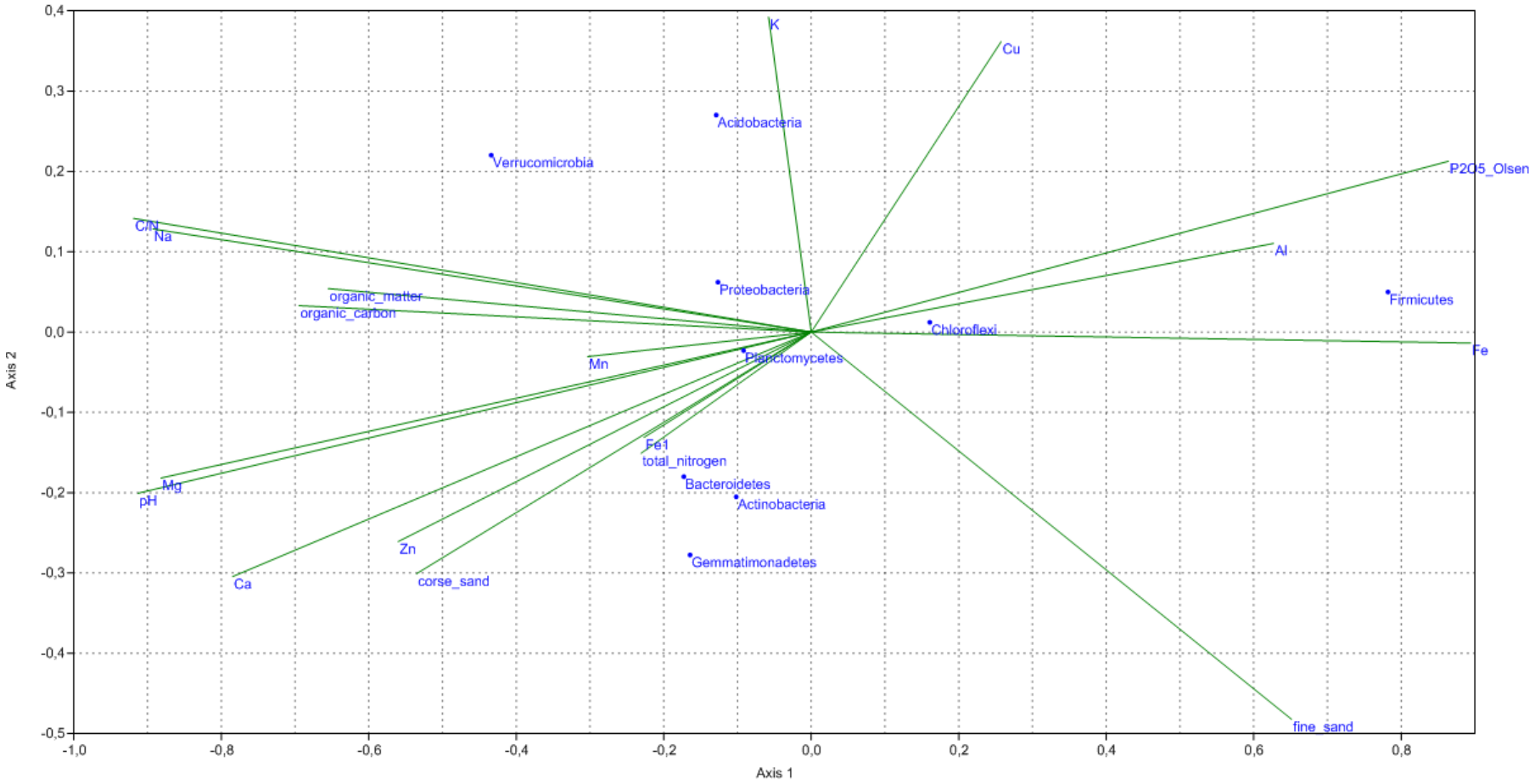

Figure 11: Triplot deriving from the canonical correspondence analysis between properties of soil and number of bacteria assigned to each phylum

Thus, these organisms may prosper in soils that are rich in C and N. *Bacillaceae* bacteria have been also isolated from gut and feces suggesting that these energy rich environments may represent suitable habitats for sporeforms *Firmicutes*. Indeed it has been suggested that spores of *Bacillaceae* (especially *Bacillus*) can grow and go through another sporulation/germination cycle inside the gut. It is therefore possible that the high relative abundance of *Firmicutes* in intensively grazed samples can be also explained by their presence/multiplication in feces of grazing sheeps. Chu et al. (2007) reported evidence for the selection of *Bacillaceae* in soils that were treated with organic manure. This suggested that members of the *Bacillaceae* respond to the addition of organic C and N sources added to soils being involved in organic matter degradation. Bardgett et al. (2001) reported an inverse relation between grazing intensities and microbial diversity and richness. Our study confirmed such as observation. However, the hypothesis advanced by Bardgett was that intensively grazed samples promote stressful conditions in soil and this may select some bacteria over others so pushing the soil microbiota toward a diversity reduction or in other terms a compositional and functional specialization. However, our taxonomic analysis did not reveal any differences in the relative abundances of *Firmicutes* among treatments at order or genus level. We warn caution in taking this as a rejection of Bardgett's hypothesis as a certain degree of specialization may still be present in the active *Firmicutes* fraction that is not distinguishable with our analysis from "total" *Firmicutes* fraction present in soil. Another intriguing observation was the low distance of *Firmicutes* response variable from the P_2O_5 environmental variable in CCA triplots. This observation suggests that *Firmicutes* are abundant in P rich sites. Indeed Kuramae et al. (2011) described a similar observation in chalk soils of the Netherlands.

Chu et al. (2007) described the phosphorous solubilizing activity of *Bacilli.* These bacteria may serve as potential biofertilizers either by introduction in soil that are currently deprived of them or when they are present by increasing their relative abundances.

Acnowledgments

We wish to acknowledge the collaboration of Prof. Roggero, dr Seddaiu; dr Roberto Lai and dr. Antonio Pulina for sharing information on soil samples and insightful for discussion. We also thanks sig Carta Domenico and sig. Gabriele Sini for chemical-physical analysis.

References

Acosta-Martínez V., Dowd S., Sun Y., Allen V., 2005. Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biology & Biochemistry 40: 2762-2770.

Bardgett R.D., Jones A.C., Jones D.L., Kemmitt S.J., Cook R., Hobbs P.J., 2001. Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. Soil Biology & Biochemistry 33: 12-13.

Bruchem J.V., Schiere H., and Keulen H.V., 1999. Dairy farming in the Netherlands in transition towards more efficient nutrient use. Livestock production science 61: 2-3. ISSN 0301-6226-p 145-153.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Pena A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature methods 7.5:335-36.

Chu H., Lin X., Fujii T., Morimoto S., Yagi K., Zhang J., 2007. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. Soil Biology & Biochemistry, 39: 2971-2976.

Collins S.L., Knapp A.K., Briggs J.M., Blair J.M., Steinauer E.M., 1998. Modulation of Diversity by Grazing and Mowing in Native Tallgrass Prairie. Science 280: 745-747.

Edgar R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26(19) 2640-2641.

Enwall K., Nyberg K., Bertilssons S., Cederluch H., Stenström J., Halcin S., 2007. Long-term impact of fertilization an activity and composition of bacterial communities and metabolic guilds in agriculture soil. Soil Biology & Biochemistry 39: 106-115.

Hilbert D., Swift M., Detling J.K., Dyer M.I., 1981. Relative growth rates and the grazing optimization hypothesis. Oecologia 51:14-18.

Janssen P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and environmental microbiology 72.3: 1719-28.

Kuramae E.E., Yergeau E., Wong L.C., Pijl A.S., Johannes A., Kowalchuk G.A., 2011. Soil characteristics more strongly influence soil bacterial communiies than land-use type. FEMS Microbiology Ecology, 79: 12-24

Lawton J.H., Bignell D.E., Bolton B., Bloemers G.F., Eggleton P., Hammond P.M., Hodda M., Holt R.D., Larsen T.B., Mawdsley N.A., Storck N.E., Srivastava D.S., Watt A.D., 1998. Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. Nature 391: 72-76.

Mandic-Mulec I., Polanca S., Van Elsas J.K., 2015. Ecology of *Bacillaceae.* Microbiology spectrum 3(2). TBS-0017-2013.

Myrold D.D., Zeglin L.H., Jansson J.K., 2013. The potential of Metagenomic Approches for Understanding Soil Microbial Processes. Soil Science Society of American Journal 78: 3-10.

Nacke H., Thürmer A., Wollherr A., Will C., Hodac L., Herold N., Schrumpf M., Daniel R., 2011. Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. PLOS ONE 6 (2): e17000.

Naether A., Foesel B.U., Naegele V., Wurst P.K., Weinert J., Bonkowski M., AltF., Oelmann Y., Polle A., Lohaus G., GockelS., Hemp A., Linsenmair K.E., Pfeiffer S., Renner S., Schöning I., Weisser W.W., Wells K., Overmann J., Friedrich M.W., 2012. Environmental factors affect Acidobacterial communities below the subgroup level in grassland and forest soils. Applied Environmental Microbialogy, 78 (20): 7398- 406.

Parham J.A., Deng S.P., Raun W.R., Johnson G.V., 2002. Long-term cattle manure application in soil. Biology and Fertilility of Soils 38: 209-215.

Quast C., Pruess E., Yilmaz P., Gerken J., Schweer T., Peplies J., Glöcner F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research (DI): D590-6

Shange R.S., Ankumah R.O., Ibekwe A.M., Zabawa R., Dowd S.E., 2012. Distinct Soil Bacterial Communities Revealed under a Diversely Managed Agroecosystem. PLOS ONE 7 (7): e40338.

Souza R.C., Cantão M.E., Vasconcelos A.T.R., Nogueira M.A., Hungria M., 2013. Soil metagenomics reveals differences under conventional and no-tillage with crop rotation or succession. Applied soil ecology 72: 49-61.

Torsvik V., Daae F.L., Sandaa R.A., Øvreås L., 1998. Novel techniques for analyzing microbial diversity in natural and perturbed environments. Journal of Biotechnology 64: 53-62.

Wang Q., Garrity G.M., Tiedje J.M., Cole J.R., 2007. Naive classificatore bayesiano per la rapida assegnazione delle sequenze di rRNA nella nuova tassonomia batterica. Applied Environmental Microbiology, 73: doi: 10.1128/aem.00062-07.

Supplemental Materials

Taxon	IG1	IG2	WLO1	WLO2	WLU1	WLU2	WPO1	WPO2	WPU1	WPU2
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota	35	60	257	26	1	8	19	28	65	13
Other;Other	5157	6426	6580	7147	6901	6847	6328	6227	6998	6917
p__Acidobacteria;Other	143	209	83	91	380	173	120	112	70	162
p__Acidobacteria;c__Acidobacteria	310	640	248	158	760	333	378	310	118	410
p__Acidobacteria;c__Acidobacteria-2	10	12	13	13	152	87	12	3	0	8
p__Acidobacteria;c__Acidobacteria-5	18	12	5	24	13	29	12	22	15	7
p__Acidobacteria;c__Acidobacteria-6	150	58	120	338	230	363	157	421	231	251
;p__Acidobacteria;c__Chloracidobacteria	72	20	124	173	105	238	102	123	124	121
p__Acidobacteria;c__Solibacteres	244	426	115	133	280	216	171	167	106	262
p__Acidobacteria;c__iii1-8	23	8	22	26	21	35	17	24	19	39
p__Actinobacteria;Other	492	254	593	554	487	482	613	526	937	593
p__Actinobacteria;c__Acidimicrobiia	151	81	219	205	225	182	244	252	209	205
p__Actinobacteria;c__Actinobacteria	1484	1189	1660	1623	1294	1312	1678	1528	2040	1407
p__Actinobacteria;c__MB-A2-108	9	1	24	25	4	20	17	35	24	9
p__Actinobacteria;c__Thermoleophilia	1053	475	1301	1551	996	1152	1301	1470	2036	1361
p__Armatimonadetes;c__S1a-1H	6	13	19	16	7	19	23	7	12	10
p__Armatimonadetes;c__[Fimbriimonadetes]	7	9	30	16	10	15	23	15	17	10
p__Bacteroidetes;Other	10	21	38	34	19	22	38	33	14	24
p__Bacteroidetes;c__Flavobacteriia	50	11	45	42	68	54	62	83	31	45
p__Bacteroidetes;c__Sphingobacteriia	359	341	858	744	318	684	599	884	610	655
p__Chlamydiae;c__Chlamydiia	10	9	19	23	19	28	16	25	18	17
p__Chloroflexi;Other	22	64	29	22	13	31	34	16	33	15
p__Chloroflexi;c__Anaerolineae	12	8	25	28	20	21	22	27	21	15
p__Chloroflexi;c__Bljii12	26	27	36	31	20	29	53	19	47	21
p__Chloroflexi;c__Chloroflexi	9	2	24	26	8	28	18	11	31	3
p__Chloroflexi;c__Ellin6529	56	15	76	130	110	102	56	110	86	64
p__Chloroflexi;c__Ktedonobacteria	172	433	119	54	38	55	218	31	39	15
p__Chloroflexi;c__Thermobacula	27	17	40	28	19	32	31	18	25	8
p__Chloroflexi;c__Thermomicrobia	12	14	16	15	13	8	14	32	16	17
p__Cyanobacteria;Other	5	6	3	3	4	4	0	2	4	1
p__Cyanobacteria;c__Chloroplast	11	10	12	31	1	19	13	4	4	6
p__Elusimicrobia;c__Elusimicrobia	9	8	18	17	14	9	20	17	10	14
p__Fibrobacteres;c__Fibrobacteria	8	1	0	3	0	4	1	3	7	2
p__Firmicutes;Other	122	129	31	23	8	12	82	42	59	31
p__Firmicutes;c__Bacilli	5537	4302	1124	768	177	525	2741	1739	1549	1248
p__Firmicutes;c__Clostridia	60	98	5	1	0	1	53	9	19	6
p__Gemmatimonadetes;c__Gemm-1	4	1	9	9	6	15	9	22	11	5

p__Gemmatimonadetes;c__Gemmatimonadetes	43	62	138	87	42	114	124	86	111	77
p__Planctomycetes;Other	19	37	91	36	33	48	49	22	47	40
p__Planctomycetes;c__Phycisphaerae	225	202	371	209	148	222	331	180	207	185
p__Planctomycetes;c__Planctomycetia	429	397	579	497	630	608	531	378	368	404
p__Proteobacteria;Other	245	441	423	376	446	477	323	397	409	519
p__Proteobacteria;c__Alphaproteobacteria	1289	1521	1334	1511	2486	1974	1114	1511	1187	2012
p__Proteobacteria;c__Betaproteobacteria	639	940	1507	758	798	724	988	931	805	1057
p__Proteobacteria;c__Deltaproteobacteria	161	167	164	209	151	242	157	190	178	130
p__Proteobacteria;c__Gammaproteobacteria	190	221	367	158	180	196	236	255	170	223
;p__TM7;c__TM7-1	12	11	23	13	10	20	21	16	18	4
p__Tenericutes;c__Mollicutes	19	29	40	9	5	8	18	18	26	22
p__Verrucomicrobia;Other	82	44	110	251	239	209	97	185	103	179
p__Verrucomicrobia;c__Opitutae	23	12	39	18	15	15	27	25	24	12
p__Verrucomicrobia;c__[Pedosphaerae]	137	130	213	183	141	173	147	178	147	136
p__Verrucomicrobia;c__[Spartobacteria]	505	170	422	1343	1832	1581	379	1106	430	895

Table S1: Absolute counts of OTUs assigned to bacteria phyla

Eigenvectors													
Crenarchaeota	0,37017	0,08805	0,18647	0,41084	0,06648	-0,20018	-0,55277	0,09002	-0,35795	-0,03471	-0,10303	0,31998	-0,22620
Other	-0,09528	0,39435	0,02320	-0,16251	0,68672	-0,09389	-0,01976	-0,23706	0,20596	0,06196	0,18891	0,41620	0,12769
Acidobacteria	-0,40680	0,03308	0,33577	0,06302	-0,03695	0,09665	0,29011	0,51186	-0,16053	-0,07986	0,35545	0,30146	-0,33237
Actinobacteria	0,17184	0,21341	-0,53394	-0,09270	0,08603	-0,36636	0,05368	0,45574	0,00531	0,41275	0,13477	-0,18400	-0,23796
Armatimonadetes	0,33549	0,17805	0,32381	-0,32584	0,05211	0,24765	-0,22198	-0,04194	-0,11920	-0,03289	0,54471	-0,45828	-0,08733
Bacteroidetes	0,20985	0,34725	-0,20342	0,04187	-0,26050	0,62694	-0,07336	0,05432	0,40857	0,03571	-0,10779	0,26860	-0,26652
Chloroflex	0,22145	-0,22614	0,41157	-0,39314	0,20579	0,06551	-0,04605	0,46177	0,19747	0,22701	-0,44954	0,05578	0,13474
Firmicutes	0,12299	-0,48768	0,01928	-0,01593	-0,11523	0,09047	0,04229	-0,29827	-0,03415	0,64954	0,31887	0,32301	-0,07151
Gemmatimonadete	0,32861	0,32258	0,00073	-0,19398	-0,11443	0,08587	0,52016	-0,10304	-0,58933	0,04915	-0,18996	0,18743	0,16655
Planctomycetes	0,18479	0,24831	0,37937	-0,03689	-0,45052	-0,55481	0,17277	-0,14467	0,42559	-0,00455	0,07168	0,08981	-0,05479
Proteobacteria	-0,17963	0,28894	0,32640	0,48062	0,16322	0,12866	0,15746	-0,12358	0,01027	0,49624	-0,22624	-0,39715	-0,09050
Tenericutes	0,38020	-0,09773	-0,01889	0,50221	0,10523	0,09028	0,24042	0,28708	0,19515	-0,09826	0,31030	0,01050	0,53828
Verrucomicrobia	-0,34521	0,30013	0,00711	-0,08545	-0,36777	0,02828	-0,40674	0,17282	-0,12543	0,28997	0,09170	0,09489	0,57864

Table S2: Autovectors of principal component analysis

Taxon	IG1	IG2	WLO1	WLO2	WLU1	WLU2	WPO1	WPO2	WPU1	WPU2
Unclassified;Other;Other;Other	6	62	55	11	3	4	3	12	12	6
k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales	35	55	244	26	1	8	19	27	61	13
Other;Other;Other	5157	6426	6580	7147	6901	6847	6328	6227	6998	6917
p__AD3;c__ABS-6;o__	10	7	4	8	1	8	8	2	3	0
p__Acidobacteria;Other;Other	143	209	83	91	380	173	120	112	70	162
p__Acidobacteria;c__;o__	2	1	11	21	12	25	12	4	5	13
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales	310	640	248	158	760	333	378	310	118	410
p__Acidobacteria;c__Acidobacteria-2;o__	10	12	13	13	152	87	12	3	0	8
p__Acidobacteria;c__Acidobacteria-5;o__	18	12	5	24	13	29	12	22	15	7
p__Acidobacteria;c__Acidobacteria-6;Other	22	10	8	59	36	51	21	68	37	26
p__Acidobacteria;c__Acidobacteria-6;o__iii1-15	126	41	109	274	187	304	133	345	189	220
p__Acidobacteria;c__Chloracidobacteria;o__	72	20	124	173	105	238	102	123	124	121
p__Acidobacteria;c__Solibacteres;o__Solibacterales	244	426	115	133	280	216	171	167	106	262
p__Acidobacteria;c__Sva0725;o__Sva0725	8	11	45	17	20	16	46	7	13	16
p__Acidobacteria;c__TM1;o__	2	1	0	1	0	0	0	0	0	0
p__Acidobacteria;c__iii1-8;o__32-20	4	1	1	3	7	6	1	1	3	1
p__Acidobacteria;c__iii1-8;o__DS-18	19	7	21	23	14	29	16	23	16	38
p__Actinobacteria;Other;Other	492	254	593	554	487	482	613	526	937	593
p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales	151	81	219	205	225	182	244	252	209	205
p__Actinobacteria;c__Actinobacteria;Other	25	23	39	30	35	32	28	38	46	34
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales	1459	1165	1621	1588	1256	1279	1649	1482	1992	1370
p__Actinobacteria;c__MB-A2-108;o__0319-7L14	9	1	24	25	4	20	17	35	24	8
p__Actinobacteria;c__Thermoleophilia;Other	93	21	128	150	81	97	108	131	237	155
p__Actinobacteria;c__Thermoleophilia;o__Gaiellales	353	89	505	642	311	444	440	633	785	520
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales	607	365	668	759	604	611	753	706	1014	686
p__Armatimonadetes;c__Chthonomonadetes;o__Chthonomonadales	7	19	17	17	1	10	11	7	8	5
p__Armatimonadetes;c__S1a-1H;o__	6	13	19	16	7	19	23	7	12	10
p__Bacteroidetes;Other;Other	10	21	38	34	19	22	38	33	14	24
p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales	50	11	45	42	68	54	62	82	31	44
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales	359	341	858	744	318	684	599	884	610	655
p__Chlamydiae;c__Chlamydiia;o__Chlamydiales	10	9	16	22	15	28	14	22	17	12
p__Chloroflexi;Other;Other	22	64	29	22	13	31	34	16	33	15
p__Chloroflexi;c__Anaerolineae;o__SBR1031	3	7	13	8	3	6	10	6	7	6
p__Chloroflexi;c__Bljii12;o__AKYG885	8	4	13	16	12	9	20	10	10	13
p__Chloroflexi;c__Bljii12;o__B07_WMSP1	18	21	16	10	5	12	24	5	26	5
p__Chloroflexi;c__Chloroflexi;o__Roseiflexales	7	2	18	24	7	19	13	7	20	2
p__Chloroflexi;c__Ellin6529;o__	56	15	76	130	110	102	56	110	86	64
p__Chloroflexi;c__Ktedonobacteria;Other	22	55	15	5	8	4	27	14	12	9
p__Chloroflexi;c__Ktedonobacteria;o__B12-WMSP1	4	43	7	2	1	2	9	0	5	0
p__Chloroflexi;c__Ktedonobacteria;o__JG30-KF-AS9	21	15	5	1	0	0	11	3	1	0

p__Chloroflexi;c__Ktedonobacteria;o__Thermogemmatisporales	125	317	89	44	26	49	165	14	18	6
p__Chloroflexi;c__Thermobacula;o__Thermobaculales	27	17	40	28	19	32	31	18	25	8
p__Chloroflexi;c__Thermomicrobia;o__Ellin6537	6	14	8	2	4	2	8	7	1	7
p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45	4	0	5	13	6	6	5	22	14	10
p__Cyanobacteria;c__Chloroplast;o__Streptophyta	3	3	9	31	1	18	10	3	3	4
p__Elusimicrobia;c__Elusimicrobia;o__FAC88	6	4	11	10	8	4	10	14	6	10
p__Elusimicrobia;c__Elusimicrobia;o__IIb	1	3	5	4	3	2	8	3	2	2
p__Fibrobacteres;c__Fibrobacteria;o__258ds10	8	1	0	3	0	4	1	3	7	2
p__Firmicutes;Other;Other	122	129	31	23	8	12	82	42	59	31
p__Firmicutes;c__Bacilli;Other	4001	3032	743	513	89	347	1878	1164	1070	823
p__Firmicutes;c__Bacilli;o__	7	5	1	1	8	3	5	7	2	1
p__Firmicutes;c__Bacilli;o__Bacillales	1440	1201	363	244	78	172	822	523	458	389
p__Firmicutes;c__Bacilli;o__Exiguobacterales	74	41	13	9	2	3	25	43	18	34
p__Firmicutes;c__Bacilli;o__Turicibacterales	15	22	1	1	0	0	9	1	1	1
p__Firmicutes;c__Clostridia;o__Clostridiales	59	97	5	1	0	1	53	9	18	6
p__Gemmatimonadetes;c__Gemmatimonadetes;Other	12	33	25	21	8	20	39	14	31	27
p__Gemmatimonadetes;c__Gemmatimonadetes;o__	8	1	27	13	8	20	17	20	28	17
p__Gemmatimonadetes;c__Gemmatimonadetes;o__Ellin5290	8	16	43	21	18	31	24	36	20	17
p__Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonadales	6	5	32	16	2	24	29	8	23	2
p__Gemmatimonadetes;c__Gemmatimonadetes;o__N1423WL	9	7	9	12	4	15	12	5	6	13
p__Nitrospirae;c__Nitrospira;o__Nitrospirales	1	5	5	12	0	12	4	12	6	7
p__OD1;c__ZB2;o__	2	4	20	4	0	4	1	1	5	0
p__Planctomycetes;Other;Other	19	37	91	36	33	48	49	22	47	40
p__Planctomycetes;c__Phycisphaerae;o__	220	200	357	201	142	213	325	177	198	181
p__Planctomycetes;c__Phycisphaerae;o__Phycisphaerales	5	2	12	6	3	7	2	1	5	3
p__Planctomycetes;c__Pla4;o__	4	0	2	3	0	11	2	4	0	0
p__Planctomycetes;c__Planctomycetia;Other	28	39	79	69	53	74	66	39	51	47
p__Planctomycetes;c__Planctomycetia;o__Gemmatales	281	298	372	303	459	395	372	187	221	208
p__Planctomycetes;c__Planctomycetia;o__Pirellulales	99	42	121	112	89	110	87	127	93	128
p__Planctomycetes;c__Planctomycetia;o__Planctomycetales	21	18	6	10	26	23	5	24	3	21
p__Proteobacteria;Other;Other	245	441	423	376	446	477	323	397	409	519
p__Proteobacteria;c__Alphaproteobacteria;Other	134	258	189	240	375	315	145	224	195	329
p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales	61	97	86	69	182	90	71	159	77	170
p__Proteobacteria;c__Alphaproteobacteria;o__Ellin329	76	64	100	45	101	86	83	86	47	88
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales	533	668	513	701	1232	912	378	612	509	869
p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales	409	309	231	339	495	374	290	265	222	378
p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales	10	12	23	11	0	4	7	14	33	11
p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales	64	111	186	94	89	176	138	137	100	162
p__Proteobacteria;c__Betaproteobacteria;Other	124	221	401	228	203	167	243	231	251	292
p__Proteobacteria;c__Betaproteobacteria;o__A21b	15	18	57	26	18	29	49	27	23	32
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales	329	522	673	311	419	326	436	482	349	548

p__Proteobacteria;c__Betaproteobacteria;o__Ellin6067	68	51	231	83	59	102	114	63	59	75
p__Proteobacteria;c__Betaproteobacteria;o__MND1	28	22	34	40	21	42	25	34	29	26
p__Proteobacteria;c__Betaproteobacteria;o__SC-I-84	70	103	100	63	72	51	120	83	91	76
p__Proteobacteria;c__Deltaproteobacteria;Other	15	9	10	21	17	15	18	14	20	10
p__Proteobacteria;c__Deltaproteobacteria;o__MIZ46	10	11	17	3	3	8	4	0	10	0
p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales	112	111	100	136	98	143	97	117	112	103
p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales	14	25	27	27	25	50	28	39	21	9
p__Proteobacteria;c__Gammaproteobacteria;Other	23	28	63	11	23	38	42	22	33	29
p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales	3	7	40	15	6	5	22	8	8	1
p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales	151	176	207	107	143	145	149	206	120	169
p__TM7;c__TM7-1;o__	12	11	23	13	10	20	21	16	18	4
p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales	19	29	40	9	5	8	18	18	26	22
p__Verrucomicrobia;Other;Other	82	44	110	251	239	209	97	185	103	179
p__Verrucomicrobia;c__Opitutae;o__Opitutales	22	12	38	16	15	15	23	24	24	12
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales]	137	129	213	183	139	170	147	177	145	136
p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales]	505	170	422	1343	1832	1581	379	1106	430	895
p__WPS-2;c__;o__	14	42	5	3	5	4	13	3	2	6

Table S3: Absolute counts of OTUs assigned to bacteria classes

Taxon	IG1	IG2	WLO1	WLO2	WLU1	WLU2	WPO1	WPO2	WPU1	WPU2
Unclassified;Other;Other;Other;Other	6	62	55	11	3	4	3	12	12	6
p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae	35	55	244	26	1	8	19	27	61	13
Other;Other;Other;Other	5157	6426	6580	7147	6901	6847	6328	6227	6998	6917
p__AD3;c__ABS-6;o__;f__	10	7	4	8	1	8	8	2	3	0
p__Acidobacteria;Other;Other;Other	143	209	83	91	380	173	120	112	70	162
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;Other	75	213	76	47	175	63	111	95	44	109
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;f__Acidobacteriaceae	102	157	45	41	350	115	72	97	27	153
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;f__Koribacteraceae	133	270	127	70	235	155	195	118	47	148
p__Acidobacteria;c__Acidobacteria-2;o__;f__	10	12	13	13	152	87	12	3	0	8
p__Acidobacteria;c__Acidobacteria-5;o__;f__	18	12	5	24	13	29	12	22	15	7
p__Acidobacteria;c__Acidobacteria-6;Other;Other	22	10	8	59	36	51	21	68	37	26
p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__	109	36	96	243	168	264	116	277	161	184
p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__RB40	11	3	4	13	12	19	7	38	13	27
p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__mb2424	1	0	3	10	4	15	5	18	11	2
p__Acidobacteria;c__Chloracidobacteria;o__;f__	72	20	124	173	105	238	102	123	124	121
p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae	244	426	115	133	280	216	171	167	106	262
p__Acidobacteria;c__Sva0725;o__Sva0725;f__	8	11	45	17	20	16	46	7	13	16
p__Acidobacteria;c__iii1-8;o__DS-18;f__	19	7	21	23	14	29	16	23	16	38
p__Actinobacteria;Other;Other;Other	492	254	593	554	487	482	613	526	937	593
p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;Other	105	68	149	121	144	113	163	156	157	116
p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111	18	6	24	31	22	21	35	32	33	32
p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__EB1017	25	3	40	51	58	46	37	55	11	51
p__Actinobacteria;c__Actinobacteria;Other;Other	25	23	39	30	35	32	28	38	46	34
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other	697	636	658	709	612	540	793	643	853	713
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Catenulisporaceae	60	41	28	6	9	5	37	13	9	3
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae	37	5	13	11	5	6	30	25	15	26
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Frankiaceae	41	8	56	28	31	33	47	30	42	30
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae	65	66	114	87	23	44	100	50	164	21
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae	26	16	17	11	8	3	41	18	27	9
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae	11	17	10	35	6	12	11	9	8	6
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae	18	39	69	21	20	17	47	49	36	33
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae	111	85	133	119	74	131	115	113	155	98
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae	111	106	168	146	203	110	148	142	199	124
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nakamurellaceae	6	7	5	5	2	4	4	5	1	17
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae	73	11	115	107	39	76	76	92	129	97
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae	35	32	40	55	73	57	34	51	42	31
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Sporichthyaceae	4	1	10	8	5	9	11	9	7	6
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae	129	63	121	201	118	186	121	161	246	112
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Thermomonosporaceae	21	17	28	16	11	22	15	21	34	20
p__Actinobacteria;c__MB-A2-108;o__0319-7L14;f__	9	1	24	25	4	20	17	35	24	8

p__Actinobacteria;c__Thermoleophilia;Other;Other	93	21	128	150	81	97	108	131	237	155
p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;Other	17	8	29	41	26	30	33	46	42	34
p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__	17	5	25	14	12	9	23	14	13	7
p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae	319	76	451	587	271	405	384	570	730	477
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;Other	222	105	333	382	251	306	288	295	494	301
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__	226	29	173	221	185	183	226	277	310	241
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Conexibacteraceae	115	215	78	82	104	61	169	61	55	46
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Patulibacteraceae	10	1	49	27	4	16	35	24	59	20
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae	34	15	35	47	60	45	35	49	96	78
p__Armatimonadetes;Other;Other;Other	4	3	7	3	0	5	7	1	6	1
p__Armatimonadetes;c__Chthonomonadetes;o__Chthonomonadales;f__Chthonomonadaceae	7	19	17	17	1	10	11	7	8	5
p__Armatimonadetes;c__S1a-1H;o__;f__	6	13	19	16	7	19	23	7	12	10
p__Armatimonadetes;c__[Fimbriimonadetes];o__[Fimbriimonadales];f_[Fimbriimonadaceae]	4	5	12	11	3	10	10	7	5	6
p__Bacteroidetes;Other;Other;Other	10	21	38	34	19	22	38	33	14	24
p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae	50	10	45	42	68	54	62	81	31	44
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;Other	28	22	96	59	13	42	62	62	49	34
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__	8	4	22	28	7	25	13	29	19	13
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Chitinophagaceae	263	243	618	591	212	531	428	686	487	525
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Flammeovirgaceae	15	2	10	37	5	45	11	44	36	18
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Flexibacteraceae	8	11	8	11	5	6	19	18	8	11
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae	37	59	100	17	76	34	66	44	11	53
p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;Other	7	2	4	7	7	12	3	7	2	4
p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Parachlamydiaceae	3	6	12	15	8	15	11	15	15	8
p__Chloroflexi;Other;Other;Other	22	64	29	22	13	31	34	16	33	15
p__Chloroflexi;c__Anaerolineae;o__Caldilineales;f__Caldilineaceae	8	1	4	2	3	4	7	13	8	1
p__Chloroflexi;c__Bljii12;o__B07_WMSP1;Other	11	14	6	2	3	7	13	3	16	2
p__Chloroflexi;c__Bljii12;o__B07_WMSP1;f__FFCH4570	7	7	7	6	2	4	10	1	7	1
p__Chloroflexi;c__Chloroflexi;o__Roseiflexales;f__Kouleothrixaceae	7	2	18	24	7	19	11	5	19	1
p__Chloroflexi;c__Ellin6529;o__;f__	56	15	76	130	110	102	56	110	86	64
p__Chloroflexi;c__Ktedonobacteria;Other;Other	22	55	15	5	8	4	27	14	12	9
p__Chloroflexi;c__Ktedonobacteria;o__B12-WMSP1;f__	4	43	7	2	1	2	9	0	5	0
p__Chloroflexi;c__Ktedonobacteria;o__JG30-KF-AS9;f__	21	15	5	1	0	0	11	3	1	0
p__Chloroflexi;c__Ktedonobacteria;o__Thermogemmatisporales;Other	8	24	11	4	7	6	20	1	7	0
p__Chloroflexi;c__Ktedonobacteria;o__Thermogemmatisporales;f__	23	54	14	11	4	9	16	2	3	2
p__Chloroflexi;c__Ktedonobacteria;o_Thermogemmatisporales;f_Thermogemmatisporaceae	94	239	64	29	15	34	129	11	8	4
p__Chloroflexi;c__Thermobacula;o__Thermobaculales;f__Thermobaculaceae	27	17	40	28	19	32	31	18	25	8
p__Chloroflexi;c__Thermomicrobia;o__Ellin6537;f__	6	14	8	2	4	2	8	7	1	7
p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45;f__	4	0	5	13	6	6	5	22	14	10
p__Cyanobacteria;Other;Other;Other	5	6	3	3	4	4	0	2	4	1
p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__	3	3	9	31	1	18	10	3	3	4
p__Elusimicrobia;c__Elusimicrobia;o__FAC88;f__	6	4	11	10	8	4	10	14	6	10

p__Fibrobacteres;c__Fibrobacteria;o__258ds10;f__	8	1	0	3	0	4	1	3	7	2
p__Firmicutes;Other;Other;Other	122	129	31	23	8	12	82	42	59	31
p__Firmicutes;c__Bacilli;Other;Other	4001	3032	743	513	89	347	1878	1164	1070	823
p__Firmicutes;c__Bacilli;o__Bacillales;Other	560	483	120	77	15	42	289	193	144	129
p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae	512	506	175	121	25	90	377	218	205	183
p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae	77	85	46	35	34	35	41	56	55	46
p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae	284	121	16	10	3	3	115	52	39	28
p__Firmicutes;c__Bacilli;o__Exiguobacterales;f__	69	37	13	7	2	3	24	43	18	32
p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae	15	22	1	1	0	0	9	1	1	1
p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	17	12	2	0	0	1	11	1	5	2
p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae	32	81	2	1	0	0	39	7	12	4
p__Gemmatimonadetes;c__Gemm-1;o__;f__	4	1	9	9	6	15	9	22	11	5
p__Gemmatimonadetes;c__Gemmatimonadetes;Other;Other	12	33	25	21	8	20	39	14	31	27
p__Gemmatimonadetes;c__Gemmatimonadetes;o__;f__	8	1	27	13	8	20	17	20	28	17
p__Gemmatimonadetes;c__Gemmatimonadetes;o__Ellin5290;f__	8	16	43	21	18	31	24	36	20	17
p__Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonadales;f__Ellin5301	6	5	29	16	1	22	28	7	23	2
p__Gemmatimonadetes;c__Gemmatimonadetes;o__N1423WL;f__	9	7	9	12	4	15	12	5	6	13
p__Planctomycetes;Other;Other;Other	19	37	91	36	33	48	49	22	47	40
p__Planctomycetes;c__Phycisphaerae;o__;f__	220	200	357	201	142	213	325	177	198	181
p__Planctomycetes;c__Planctomycetia;Other;Other	28	39	79	69	53	74	66	39	51	47
p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae	147	139	257	210	168	248	239	121	115	108
p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae	127	146	100	92	274	140	123	62	97	94
p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae	98	42	120	112	89	106	86	126	92	127
p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae	21	18	6	10	26	23	5	24	3	21
p__Proteobacteria;Other;Other;Other	245	441	423	376	446	477	323	397	409	519
p__Proteobacteria;c__Alphaproteobacteria;Other;Other	134	258	189	240	375	315	145	224	195	329
p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;Other	8	22	9	12	27	13	12	22	7	23
p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae	52	75	76	57	154	77	57	136	70	147
p__Proteobacteria;c__Alphaproteobacteria;o__Ellin329;f__	76	64	100	45	101	86	83	86	47	88
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other	268	450	313	452	799	531	248	348	323	521
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae	83	109	66	49	113	98	42	76	59	103
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae	128	61	95	147	201	202	64	145	76	184
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae	5	14	7	13	0	8	11	2	17	3
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae	21	19	12	9	86	26	5	9	10	24
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae	8	2	2	8	9	12	3	14	11	3
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	12	4	13	15	11	5	2	10	4	15
p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;Other	39	28	27	28	71	45	23	34	21	54
p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae	131	105	47	48	107	53	78	42	50	72
p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae	238	176	155	259	317	275	189	185	151	252
p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae	59	106	178	88	84	168	132	125	91	150
p__Proteobacteria;c__Betaproteobacteria;Other;Other	124	221	401	228	203	167	243	231	251	292

p__Proteobacteria;c__Betaproteobacteria;o__A21b;f__EB1003	12	8	16	13	16	18	20	21	12	18
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;Other	35	80	56	34	34	29	54	43	44	62
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae	125	222	202	63	216	66	91	163	57	280
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	157	205	396	203	156	223	277	253	238	198
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae	12	15	17	11	12	8	12	18	10	8
p__Proteobacteria;c__Betaproteobacteria;o__Ellin6067;f__	68	51	231	83	59	102	114	63	59	75
p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__	28	22	34	40	21	42	25	34	29	26
p__Proteobacteria;c__Betaproteobacteria;o__SC-I-84;f__	70	103	100	63	72	51	120	83	91	76
p__Proteobacteria;c__Deltaproteobacteria;Other;Other	15	9	10	21	17	15	18	14	20	10
p__Proteobacteria;c__Deltaproteobacteria;o__FAC87;f__	4	0	4	2	2	4	1	0	0	1
p__Proteobacteria;c__Deltaproteobacteria;o__MIZ46;f__	10	11	17	3	3	8	4	0	10	0
p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;Other	43	48	38	48	43	54	43	46	37	42
p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__	18	30	25	29	19	28	14	31	21	29
p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae	18	5	10	16	16	22	8	19	24	11
p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae	14	7	12	24	7	10	19	7	14	12
p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Polyangiaceae	18	16	6	15	10	18	9	12	12	8
p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae	14	25	27	26	24	49	28	39	21	9
p__Proteobacteria;c__Gammaproteobacteria;Other;Other	23	28	63	11	23	38	42	22	33	29
p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae	1	5	50	0	0	0	13	2	4	1
p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae	50	36	26	32	53	62	24	60	38	55
p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae	98	126	180	74	88	82	120	144	80	113
p__TM7;c__TM7-1;o__;f__	12	11	23	13	10	20	21	16	18	4
p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae	19	29	40	9	5	8	18	18	26	22
k__Bacteria;p__Verrucomicrobia;Other;Other;Other	82	44	110	251	239	209	97	185	103	179
p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae	21	10	38	15	13	15	23	24	24	11
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];Other	69	51	98	88	58	91	79	84	66	69
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__	15	19	24	25	12	11	15	9	15	12
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__Ellin515	23	27	33	23	33	23	27	34	20	23
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__Ellin517	15	4	26	24	15	25	7	29	25	10
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__[Pedosphaeraceae]	12	25	29	19	18	20	19	20	15	20
p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];Other	6	5	13	19	46	46	12	29	11	29
p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae]	499	165	409	1324	1786	1535	367	1077	419	866
p__WPS-2;c__;o__;f__	14	42	5	3	5	4	13	3	2	6

Table S4: Absolute counts of OTUs assigned to bacterial orders

Taxon	IG1	IG2	WLO 1	WPO 1	WLO 2	WPU 1	WPO 2	WLU 1	WLU 2	WPU 2
Unclassified;Other;Other;Other;Other;Other	6	62	55	3	11	12	12	3	4	6
k_Archaea;p_Crenarchaeota;c__Thaumarchaeota;o_Nitrososphaerales;f__Nitrososphaeraceae;Other	11	16	66	5	2	17	7	0	3	4
k_Archaea;p_Crenarchaeota;c_Thaumarchaeota;o_Nitrososphaerales;f_Nitrososphaeraceae;g_Candidatus Nitrososphaera	24	35	163	11	17	38	17	1	5	7
Other;Other;Other;Other;Other	5157	6426	6580	6328	7147	6998	6227	6901	6847	6917
p__AD3;c__ABS-6;o__;f__;g__	10	7	4	8	8	3	2	1	8	0
p__Acidobacteria;Other;Other;Other;Other	143	209	83	120	91	70	112	380	173	162
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;Other;Other	75	213	76	111	47	44	95	175	63	109
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;f__Acidobacteriaceae;Other	45	78	14	27	14	9	38	158	43	65
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;f__Acidobacteriaceae;g__	24	30	8	12	2	6	10	60	14	25
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;f__Acidobacteriaceae;g__Edaphobacter	17	14	13	19	20	7	38	89	32	49
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;f__Acidobacteriaceae;g__Granulicella	13	16	4	2	0	2	2	28	19	5
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;f__Koribacteraceae;Other	76	150	70	111	30	26	67	116	56	84
p__Acidobacteria;c__Acidobacteria;o__Acidobacteriales;f__Koribacteraceae;g__	14	25	16	39	17	6	17	45	56	16
p_Acidobacteria;c_Acidobacteria;o_Acidobacteriales;f_Koribacteraceae;g__Candidatus Koribacter	43	95	41	45	23	15	34	74	43	48
p__Acidobacteria;c__Acidobacteria-2;o__;f__;g__	10	12	13	12	13	0	3	152	87	8
p__Acidobacteria;c__Acidobacteria-5;o__;f__;g__	18	12	5	12	24	15	22	13	29	7
p__Acidobacteria;c__Acidobacteria-6;Other;Other;Other	22	10	8	21	59	37	68	36	51	26
p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__;g__	109	36	96	116	243	161	277	168	264	184
p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__RB40;g__	11	3	4	7	13	13	38	12	19	27
p__Acidobacteria;c__Chloracidobacteria;o__;f__;g__	72	20	124	102	173	124	123	105	238	121
p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter	244	426	115	171	133	106	167	280	216	262
p__Acidobacteria;c__Sva0725;o__Sva0725;f__;g__	8	11	45	46	17	13	7	20	16	16
p__Acidobacteria;c__iii1-8;o__DS-18;f__;g__	19	7	21	16	23	16	23	14	29	38
p__Actinobacteria;Other;Other;Other;Other	492	254	593	613	554	937	526	487	482	593
p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;Other;Other	105	68	149	163	121	157	156	144	113	116
p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111;g__	18	6	24	35	31	33	32	22	21	32
p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__EB1017;g__	25	3	40	37	51	11	55	58	46	51
p__Actinobacteria;c__Actinobacteria;Other;Other;Other	25	23	39	28	30	46	38	35	32	34
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other;Other	697	636	658	793	709	853	643	612	540	713
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Catenulisporaceae;g__Catenulispora	60	41	28	37	6	9	13	9	5	3
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae;Other	30	4	10	23	9	14	20	4	5	18
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Frankiaceae;g__	40	8	55	45	28	39	30	31	33	30
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;Other	41	39	76	70	54	119	34	19	28	13
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;g__	19	23	22	21	20	25	11	2	9	7
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;Other	15	31	47	34	9	23	38	17	10	23
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;Other	65	66	95	82	84	96	75	43	92	60
p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Micromonosporaceae;g__Micromonospora	29	11	21	28	15	30	26	13	25	28
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium	111	106	168	148	146	199	142	203	110	124
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Kribbella	20	1	27	32	24	40	20	10	10	20

p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Nocardioides	36	5	58	25	60	58	52	21	45	60
p_Actinobacteria;c_Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Pseudonocardia	24	11	18	15	32	15	37	37	20	22
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae;Other	48	29	39	47	64	84	49	37	57	38
p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae;g__Streptomyces	77	27	82	72	127	145	106	81	126	73
p__Actinobacteria;c__Thermoleophilia;Other;Other;Other	93	21	128	108	150	237	131	81	97	155
p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;Other;Other	17	8	29	33	41	42	46	26	30	34
p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__;g__	17	5	25	23	14	13	14	12	9	7
p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;g__	319	76	451	384	587	730	570	271	405	477
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;Other;Other	222	105	333	288	382	494	295	251	306	301
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__;g__	226	29	173	226	221	310	277	185	183	241
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Conexibacteraceae;g__	113	209	78	166	82	52	58	100	55	45
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Patulibacteraceae;g__	10	1	48	35	27	59	24	4	16	20
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae;Other	16	2	11	14	17	24	22	23	14	30
p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae;g__	8	3	13	7	11	28	10	12	12	14
p_Actinobacteria;c_Thermoleophilia;o_Solirubrobacterales;f_Solirubrobacteraceae;g_Solirubrobacter	10	10	11	14	19	44	17	25	19	34
p_Armatimonadetes;c_Chthonomonadetes;o_Chthonomonadales;f_Chthonomonadaceae;g__Chthonomonas	7	19	17	11	17	8	7	1	10	5
p__Bacteroidetes;Other;Other;Other;Other	10	21	38	38	34	14	33	19	22	24
p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;Other	15	2	21	22	16	13	26	34	21	11
p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium	35	7	20	35	24	18	53	34	33	33
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;Other;Other	28	22	96	62	59	49	62	13	42	34
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Flammeovirgaceae;g__A4	15	2	10	11	37	36	44	5	45	18
p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__	32	40	35	24	10	7	35	70	28	41
p__Chloroflexi;Other;Other;Other;Other	22	64	29	34	22	33	16	13	31	15
p__Chloroflexi;c__Ellin6529;o__;f__;g__	56	15	76	56	130	86	110	110	102	64
p__Chloroflexi;c__Ktedonobacteria;Other;Other;Other	22	55	15	27	5	12	14	8	4	9
p__Chloroflexi;c__Ktedonobacteria;o__JG30-KF-AS9;f__;g__	21	15	5	11	1	1	3	0	0	0
p__Chloroflexi;c__Ktedonobacteria;o__Thermogemmatisporales;Other;Other	8	24	11	20	4	7	1	7	6	0
p__Chloroflexi;c__Ktedonobacteria;o__Thermogemmatisporales;f__;g__	23	54	14	16	11	3	2	4	9	2
p__Chloroflexi;c__Ktedonobacteria;o__Thermogemmatisporales;f__Thermogemmatisporaceae;Other	36	42	6	25	3	1	2	1	1	0
p__Chloroflexi;c__Ktedonobacteria;o__Thermogemmatisporales;f__Thermogemmatisporaceae;g__	58	197	58	104	26	7	9	14	33	4
p__Chloroflexi;c__Thermobacula;o__Thermobaculales;f__Thermobaculaceae;g__	27	17	40	31	28	25	18	19	32	8
p__Firmicutes;Other;Other;Other;Other	122	129	31	82	23	59	42	8	12	31
p__Firmicutes;c__Bacilli;Other;Other;Other	4001	3032	743	1878	513	1070	1164	89	347	823
p__Firmicutes;c__Bacilli;o__Bacillales;Other;Other	560	483	120	289	77	144	193	15	42	129
p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;Other	91	94	29	83	23	45	55	5	18	33
p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	421	412	146	294	98	160	163	19	72	150
p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Ammoniphilus	22	16	16	8	3	19	9	0	2	7
p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus	45	67	26	29	27	30	33	31	30	37
p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;Other	142	83	11	81	3	26	36	3	2	15
p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Sporosarcina	138	34	5	32	6	12	15	0	0	10
p__Firmicutes;c__Bacilli;o__Exiguobacterales;f__;g__	69	37	13	24	7	18	43	2	3	32

p__Planctomycetes;Other;Other;Other;Other	19	37	91	49	36	47	22	33	48	40
p__Planctomycetes;c__Phycisphaerae;o__;f__;g__	220	200	357	325	201	198	177	142	213	181
p__Planctomycetes;c__Planctomycetia;Other;Other;Other	28	39	79	66	69	51	39	53	74	47
p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae;Other	11	10	25	23	27	13	9	7	31	8
p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae;g__	106	119	172	172	149	82	81	130	186	67
p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae;g__Gemmata	30	10	60	44	34	20	31	31	31	33
p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae;Other	23	44	24	42	16	33	19	43	37	31
p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae;g__	83	84	53	64	45	40	28	165	75	43
p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae;g__Singulisphaera	20	18	21	15	30	22	15	66	28	20
p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;Other	31	9	29	29	35	29	53	37	36	46
p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__	23	21	29	16	36	20	28	26	35	38
p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__A17	8	1	3	4	11	7	11	10	8	14
p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__Pirellula	36	11	59	37	30	36	34	16	27	29
p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces	21	18	6	5	10	3	24	26	23	21
p__Proteobacteria;Other;Other;Other;Other	245	441	423	323	376	409	397	446	477	519
p__Proteobacteria;c__Alphaproteobacteria;Other;Other;Other	134	258	189	145	240	195	224	375	315	329
p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;Other	27	47	48	40	39	41	70	84	40	71
p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	13	20	18	11	10	20	46	62	24	60
p__Proteobacteria;c__Alphaproteobacteria;o__Ellin329;f__;g__	76	64	100	83	45	47	86	101	86	88
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other;Other	268	450	313	248	452	323	348	799	531	521
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;Other	74	98	61	34	40	43	61	98	84	93
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;Other	19	8	11	17	27	8	22	63	30	29
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia	13	5	3	8	2	7	16	8	4	13
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes	90	45	76	36	93	59	93	123	133	121
p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__	16	15	4	4	4	3	3	80	20	16
p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;Other;Other	39	28	27	23	28	21	34	71	45	54
p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;Other	101	88	36	57	34	41	32	77	39	52
p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__	27	16	10	17	13	7	8	29	11	16
p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;Other	39	18	29	27	38	26	35	59	55	53
p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__	196	158	120	155	218	107	148	257	217	197
p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;Other	11	25	42	33	41	30	30	27	79	32
p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Kaistobacter	24	46	103	76	31	44	63	29	45	66
p__Proteobacteria;c__Betaproteobacteria;Other;Other;Other	124	221	401	243	228	251	231	203	167	292
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;Other;Other	35	80	56	54	34	44	43	34	29	62
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;Other	53	96	92	38	32	28	70	104	40	132
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Burkholderia	36	52	24	14	24	13	66	99	21	120
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Salinispora	36	74	85	38	7	16	27	13	5	28
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;Other	94	137	291	184	129	179	185	106	135	139
p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Methylibium	37	43	68	67	51	37	54	43	64	45
p__Proteobacteria;c__Betaproteobacteria;o__Ellin6067;f__;g__	68	51	231	114	83	59	63	59	102	75
p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__;g__	28	22	34	25	40	29	34	21	42	26

p__Proteobacteria;c__Betaproteobacteria;o__SC-I-84;f__;g__	70	103	100	120	63	91	83	72	51	76
p__Proteobacteria;c__Deltaproteobacteria;Other;Other;Other	15	9	10	18	21	20	14	17	15	10
p__Proteobacteria;c__Deltaproteobacteria;o__MIZ46;f__;g__	10	11	17	4	3	10	0	3	8	0
p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;Other;Other	43	48	38	43	48	37	46	43	54	42
p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__;g__	18	30	25	14	29	21	31	19	28	29
p__Proteobacteria;c__Gammaproteobacteria;Other;Other;Other	23	28	63	42	11	33	22	23	38	29
p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;Other	88	101	149	106	56	72	92	78	75	82
p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae;g__Asteroleplasma	19	29	40	18	9	26	18	5	8	22
p__Verrucomicrobia;Other;Other;Other;Other	82	44	110	97	251	103	185	239	209	179
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];Other;Other	69	51	98	79	88	66	84	58	91	69
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__;g__	15	19	24	15	25	15	9	12	11	12
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__Ellin515;g__	23	27	33	27	23	20	34	33	23	23
p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__Ellin517;g__	15	4	26	7	24	25	29	15	25	10
p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];Other	76	37	79	86	265	107	155	325	266	137
p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];g__DA101	390	104	297	253	977	284	868	1343	1138	686

Table S5: Absolute counts of OTUs assigned to bacterial families

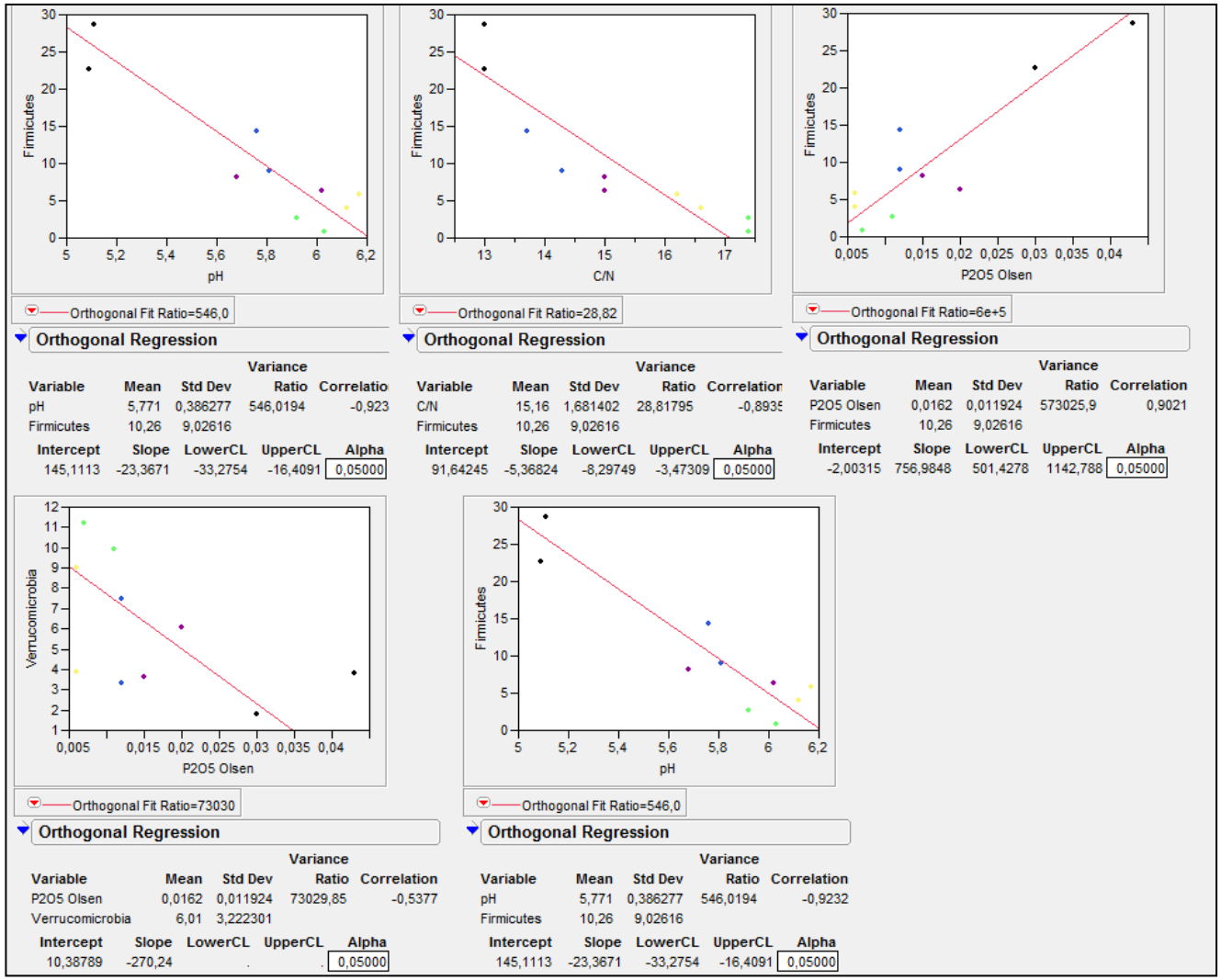

Figure S1: Regression plots between soil properties and relative abundance of bacteria at phylum level

CHAPTER 2

Comparative analysis of bacterial communities of soils treated with organic or mineral fertilization

Abstract

In the present work we analyzed the effects of organic and mineral fertilization on soil microbiota communities. The organic fertilization source was cattle manure and slurry, while the mineral was represented by a low release nitrogen fertilizer.

The experimental setup was designed to represent a typical land management system of a nitrogen vulnerable zone of central Sardinia. Our results indicated that sites receiving manure are characterized by a bacteria community enriched for species able to degrade complex organic compounds.

On the contrary, sites receiving mineral fertilization were enriched for oligotrophic species adapted to nutrients limited environments. Detailed description of the taxonomic composition of bacteria communities at class, order, family and genus level are presented. This information will serve as reference for evaluating different fertilization strategy in nitrogen vulnerable zone.

Introduction

A common view emerging from soil metagenomic analyses is that agriculture has a significant impact on richness and distribution of microbial diversity although the effects are qualitatively and quantitatively dependent on the pedological properties of the analyzed samples (Kuramae et al., 2010; Nacke et al., 2011; Shange et al., 2012).

Reports describing soil microbial assemblages in relations to land use managements have indicated the highest impacts for land forestation, a finding often associated with acidic pH. High fertilization inputs through mineral or organic inputs is considered another weighing factor. Kuramae et al. (2010) have pointed to the heavily fertilization, through mineral and organic supplies, (Bruchem et al., 1999) as a possible explanation for the high *Firmicutes* relative abundance of chalk soils in the Netherlands. Other studies have highlighted the shifts in microbial community structures following organic and inorganic amendant administration to soils (Hu et al., 2011). All these reports converge on indicating that high inputs of organic or inorganic supplies may significantly interfere with the soil microbial community structure and function.

Until the 90s, the European agriculture has been striving to achieve high competitiveness mainly through the intensification of cropping systems and the specialization of productive systems (Demurtas, 2013). As a result the number of animals bred per unit area of land has increased and this has promoted dramatic changes on the cropping and land management systems. While these improvements have in most cases covered forage needs of animals, concerns have been raised on their capability of managing the considerable amount of slurry and manure produced by animals. In the most extreme cases, specialized farming systems may have less land than theoretically required to utilize animal effluents.

N losses from intensive agriculture-farming systems are considered among the major causes of pollution of surface and ground water. An efficient N fertilization in therms of Nitrogen Use Efficiency (NUE) is considered a mandatory strategy for sustaining or increasing crop yield and quality and improving the balance of fertilizer applied and incorporated in the plant and hence removed after harvest. Crop management strategies such as a sustainable definition of the amount of fertilizer and an appropriate choice of the application route are considered crucial for NUE maximization. Moreover, all these choices and their impact in terms of NUE maximization are largely dependent on the microbial assemblage of soils.

Microbes play an important role in the cycling of N; they exclusively mediate N fixation, de/nitrification and nitrification. Nitrification is especially important in soils, because oxidation of ammonium to nitrite and nitrate ions change their charge from positive to negative. This leads to

nitrate leaching as negatively charged ions (NO_3^-) can be leached into ground water.

Therefore, the composition of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archea can dramatically influence this process. Denitrification is a microbial respiratory process during which soluble N oxides are used as electron acceptor and reduced to NO_2, NO and N_2. This occurs predominately in waterlogged areas that have become anaerobic. Because N_2 and NO are highly volatile they return to the atmosphere from soil and water. The ability to denitrify has been identified in a wide range of phylogenetically unrelated soil bacteria including members of the *Proteobacteria*, *Actinobacteria*, *Firmicutes* (Janssen et al 2006). Several classes of bacteria have been involved in the degradation of organic matter and plant litter. However many of these bacteria are not cultivable with conventional cultivation methods and thus little knowledge is to date available on their physiology. For example, Siala et al. (1973) reported that vegetative bacilli predominate the soil A1 horizon, where organic carbon is provided by plant litter and root exudates, while spore predominate in the deeper C zone. Other studies have emphasized the importance of microorganism with phosphatase and polyphosphate activity in P rich sites.

In the present work we analyzed the microbial structures community of soil fertilized with organic and inorganic fertilizers. Four fertilizer sources were analyzed: cattle manure and slurry, slurry supplied with mineral fertilizer and mineral fertilizer alone. The diversity of the soil microbial fractions were determined by analyzing the diversity of 16S rRNA sequence libraries obtained by pyrosequencing of soil metagenomic DNA. Because previous analysis of microbial soil communities have evidenced the presence of many sporulating species we analyzed also 16S RNA libraries obtained from retro-transcribed RNA extracted from the same soil samples. This latter strategy is frequently employed to identify the 'active' fraction of microbes in a environmental samples. Our results indicating that the relative abundances of *Firmicutes* and *Proteobacteria* are associated to the C/N ratio of the fertilizer sources.

Materials and Methods

Study area

Soil samples were collected within a dairy-cattle farm located in Arborea (39° 47' N 8°33' E, 3m asl). The area is characterized by a Mediterranean climate with long, hot dry summers and short mild rainy winters: the average precipitation is 600 mm with an average temperature of 17 °C. The soil is classified as Psammentic Palexeralfs (USDA, 2006). The soil texture is 94% sand with bulk density of 1,5 g cm^{-3}.

The experiment was conducted between June and September 2011. The experimental field is represented in figure 1.

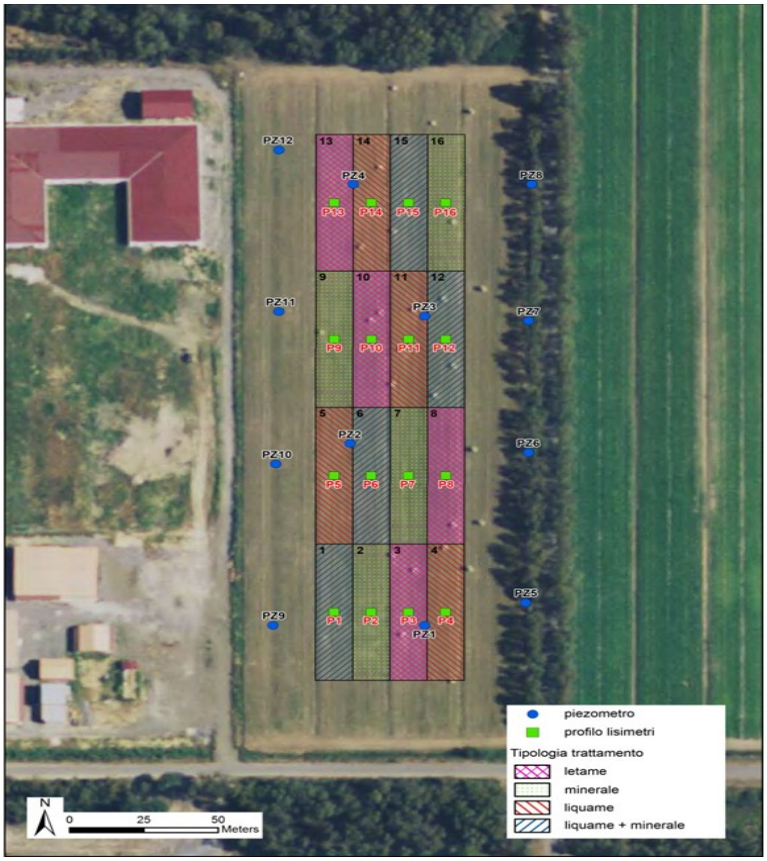

Figure 1: Aereal photo of sampling sites in Arborea

The crop rotation was based on a double cropping systems with hay crop. The fertilization treatments were as follow:

- Manure (MA): cattle farmyard manure applied before the sowing of each crop with a conventional spreader. About 70% of total dose was applied to maize at the end of maize and the remaining to hay crop in October.
- Slurry (SL): cattle slurry applied before sowing with a conventional spreader.
- Slurry and Mineral (SM): the slurry was applied at a corresponding target rate of 100 and 70 kg ha^{-1} for maize and ryegrass respectively and mineral fertilizer ENTEC26® (ammonium nitrogen stabilized by the inhibitor of nitrification 3,4 dimethyl pyrazole phosphate) at a rate of 216 and 60 kg ha^{-1} N applied before sowing for maize at the end of hay crop tillering, respectively.
- Mineral (MI) mineral fertilizer applied before sowing for maize and for hay crop. The

mineral was ENTEC 26 at a rate of 316 and 130 kg ha^{-1} N applied before sowing for maize at the end of hay crop tillering, respectively.

Nucleic acid extraction, PCR amplification and 16 S rRNA library sequencing

Total genomic DNA and total RNA extraction from soils was performed with the RNA PowerSoil™Total Isolation Kit (Mobio Laboratories, inc., corporate headquarters 2746 Loker Avenue West) according to manufacturer's instructions.

The quality and concentrations of DNA and RNA solution were checked with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Goettingen, GE). The RNA samples were subjected to DNase treatment to remove eventual contamination of genomic DNA. The absence of residual genomic DNA after DNase treatment was verified by PCR amplification with 16S ribosomal gene specific primers. The total RNA (up to 5 µg) was retrotranscribed with Bac907f Primer (CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCGTCAATTCCTTTRAGTTT-10µM) and dNTP mixture containing 10 mM of each of the four deoxynucleotide triphosphates. The 5X reaction buffer, the 0.1 M DDT, the RiboLock RNase Inhibitor (Fermentas, #EO0381) and the SuperScript™ III RT (200U) were added. The generated cDNA as reported in the manufacturer's instructions.

Genomic DNA and cDNA samples were amplified by PCR with 1 µl dNTPs (10 mM each) and the BAC907f primer reverse with the initial denaturation at 98°C, 25 cycles of denaturation at 98°C for 30", annealing at 62°C for 30" following by extension at 72°C for 30". Primers (10µM) forward for amplification as well as 454 adaptors whit the unique MIDs for each sample are listed in table 1. The amplification products were displayed in TAE 1X agarose gel. The concentrations of amplification products were determined with the Qubit fluorometer (Invitrogen) as recommende by the manufacturer. The Gottingen Genomics Laboratory determined the sequences of the 16S rRNA by using a Roche GS-FLX 454 Pyrosequencer.

Dataset analyses

Sequence reads were subjected to quality filter with the python script implemented in Qiime (Caporaso et al., 2010). The sequences were assigned to samples/treatments based on the MID sequences and using the python scrip split.libraries.py.

Sample ID	Barcode Sequence	Linker Primer
Pkl 189 (DNA)	**ACGAGTGCGT**	TCACGRCACGA
Pkl 191 (DNA)	**AGCACTGTAG**	TCACGRCACGA
Pkl 192 (DNA)	**ATCAGACACG**	TCACGRCACGA
Pkl 193 (DNA)	**ATATCGCGAG**	TCACGRCACGA
Pkl 195 (DNA)	**CTCGCGTGTC**	TCACGRCACGA
Pkl 196 (DNA)	**TCTCTATGCG**	TCACGRCACGA
Pkl 197 (DNA)	**TGATACGTCT**	TCACGRCACGA
Pkl 198 (DNA)	**CATAGTAGTG**	TCACGRCACGA
Pkl 201 (DNA)	**TCACGTACTA**	TCACGRCACGA
Pkl 202 (DNA)	**CGTCTAGTAC**	TCACGRCACGA
Pkl 204 (DNA)	**TGTACTACTC**	TCACGRCACGA
Pkl 207 (DNA)	**TACGAGTATG**	TCACGRCACGA
Pkl 208 (DNA)	**TACTCTCGTG**	TCACGRCACGA
Pkl 211 (DNA)	**ACATACGCGT**	TCACGRCACGA
Pkl 212 (DNA)	**ACGCGAGTAT**	TCACGRCACGA
Pkl 214 (DNA)	**ACTGTACAGT**	TCACGRCACGA
Pkl 236 (cDNA)	**AGCTCACGTA**	TCACGRCACGA
Pkl 237 (cDNA)	**AGTATACATA**	TCACGRCACGA
Pkl 238 (cDNA)	**AGTCGAGAGA**	TCACGRCACGA
Pkl 239 (cDNA)	**AGTGCTACGA**	TCACGRCACGA
Pkl 241 (cDNA)	**CGCAGTACGA**	TCACGRCACGA
Pkl 242 (cDNA)	**CGCGTATACA**	TCACGRCACGA
Pkl 243 (cDNA)	**CGTACAGTCA**	TCACGRCACGA
Pkl 244 (cDNA)	**CGTACTCAGA**	TCACGRCACGA
Pkl 245 (cDNA)	**CTACGCTCTA**	TCACGRCACGA
Pkl 246 (cDNA)	**CTATAGCGTA**	TCACGRCACGA
Pkl 247 (cDNA)	**TACGTCATCA**	TCACGRCACGA
Pkl 248 (cDNA)	**TAGTCGCATA**	TCACGRCACGA
Pkl 249 (cDNA)	**TATATATACA**	TCACGRCACGA
Pkl 250 (cDNA)	**TATGCTAGTA**	TCACGRCACGA
Pkl 251 (cDNA)	**TCACGCGAGA**	TCACGRCACGA

Table 1: List of the sequence of the primers

OTU picking was carried out using the phyton script otu.picking.py (Caporaso et al., 2010). This script uses uclust as clustering procedure. Taxonomic classification of OTUs was performed by BLAST homology search against the SILVA database (Quast et al., 2013) of 16s rRNA sequences.

Alpha diversity indexes were calculated with Qiime (Caporaso et al., 2010). The analyses of metagenomic coverage were carried out by a rarefaction based approach as implemented in Qiime. Briefly, samples of a given number of sequences were randomly sampled from each dataset. The number of extracted sequence increased progressively by a factor of one hundred up to a final count

of 2000. For each random sample the chao1 index and the number of species was determined using alpha.diversity.py script. The alpha diversity values were then collected together before graphic visualization. A sequencing depth of 1500 was used for further analysis.

Principal component analysis was performed with the JMP software (SAS Institute Inc., Cary, NC, 1989-2007).

Differentiation of bacterial communities among samples was tested with ANOSIM as implemented in QIIME (Caporaso et al., 2010).

Results

The soil samples were collected from maize fields subjected to different fertilization managements (Demurtas, 2013). The treatments were differentiated for the fertilization source but not for the total level of nitrogen administered, that in all cases was 316 kg N ha^{-1}.

For a complete description of fertilizer sources and analyzed samples see table 2.

The MA samples were taken from maize plot fertilized with cattle farmyard manure. The fertilizer source for the SL samples was cattle slurry. The mineral fertilizer of M treatments was ENTEC 26®. A mix of mineral fertilizer and slurry was the fertilizer source for SM samples. In all cases the fertilizer were administered according to the prescriptions detailed in Directive 91/676 EEC for NVZ regions.

Bacterial alpha diversity

The relations between sequencing surveying effort and taxonomic diversity were analyzed by a rarefaction based approach at a similarity distance of 3% and 20%. The Chao1 and number of otus index were used to estimate bacterial richness. As shown in figure 2a and 2b at 3% of sequence similarity distance none of the curves reached saturation indicating that at this phylogenetic distance our datasets cannot be considered an exhaustive representation of the full extent of bacterial diversity of analyzed soils.

On the contrary, at a similarity distance of 20% most curves reached saturation (see figure S1a/S1b). Thus, at this genetic distance, the datasets represent the full extent of soil bacterial diversity observed. All subsequent analyses were carried out considering a surveying effort of 1500 sequences per sample. Comparison of the rarefaction analyses with the number of species determined by Chao1 richness indicated that 45% and 90% of taxonomic richness was covered at this sampling depth.

Name Sample	Type	Data Collection	Description
gMA_1	Genomic DNA	20.09.2011	Manure
gMA_2	Genomic DNA	20.09.2011	Manure
gMA_3	Genomic DNA	20.09.2011	Manure
gMA_4	Genomic DNA	20.09.2011	Manure
gSLM_1	Genomic DNA	20.09.2011	slurry+mineral
gSLM_2	Genomic DNA	20.09.2011	slurry+mineral
gSLM_3	Genomic DNA	20.09.2011	slurry+mineral
gSLM_4	Genomic DNA	20.09.2011	slurry+mineral
gSL_1	Genomic DNA	20.09.2011	Slurry
gSL_2	Genomic DNA	20.09.2011	Slurry
gSL_3	Genomic DNA	20.09.2011	Slurry
gSL_4	Genomic DNA	20.09.2011	Slurry
gM_1	Genomic DNA	20.09.2011	Mineral
gM_2	Genomic DNA	20.09.2011	Mineral
gM_3	Genomic DNA	20.09.2011	Mineral
gM_4	Genomic DNA	20.09.2011	Mineral
cMA_1	cDNA	20.09.2011	Manure
cMA_2	cDNA	20.09.2011	Manure
cMA_3	cDNA	20.09.2011	Manure
cMA_4	cDNA	20.09.2011	Manure
cSLM_1	cDNA	20.09.2011	slurry+mineral
cSLM_2	cDNA	20.09.2011	slurry+mineral
cSLM_3	cDNA	20.09.2011	slurry+mineral
cSLM_4	cDNA	20.09.2011	slurry+mineral
cSL_1	cDNA	20.09.2011	Slurry
cSL_2	cDNA	20.09.2011	Slurry
cSL_3	cDNA	20.09.2011	Slurry
cSL_4	cDNA	20.09.2011	Slurry
cM_1	cDNA	20.09.2011	Mineral
cM_2	cDNA	20.09.2011	Mineral
cM_3	cDNA	20.09.2011	Mineral
cM_4	cDNA	20.09.2011	Mineral
cMA_1	cDNA	20.09.2011	Manure
cMA_2	cDNA	20.09.2011	Manure
cMA_3	cDNA	20.09.2011	Manure
cMA_4	cDNA	20.09.2011	Manure

Table 2: Description of fertilizer sources for each samples

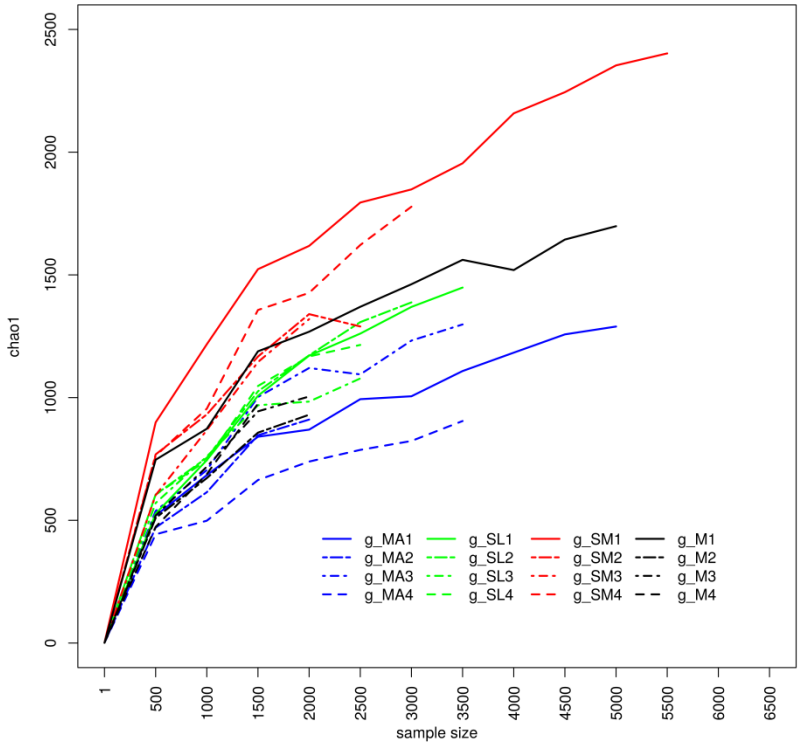

Figure 2a Rarefaction curve (chao1) at a sequence similarity level of 3%

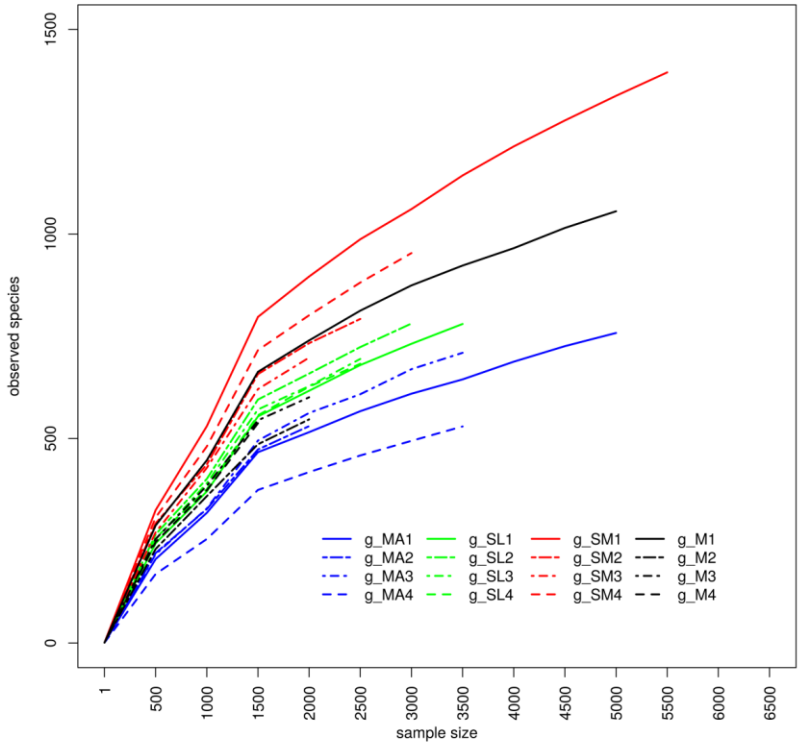

Figure 2b: Rarefaction curve (observed species) at a sequence similarity level of 3%

The values of diversity indexes calculated for genomic and cDNA datasets were similar (Figure 3a/ 3b). An exception was observed for the MA treatment that showed lower diversity indexes compared to corresponding cDNA samples. The Shannon indexes did not reveal significant differences between samples (see table S1a for 3% and S1b for 20%).

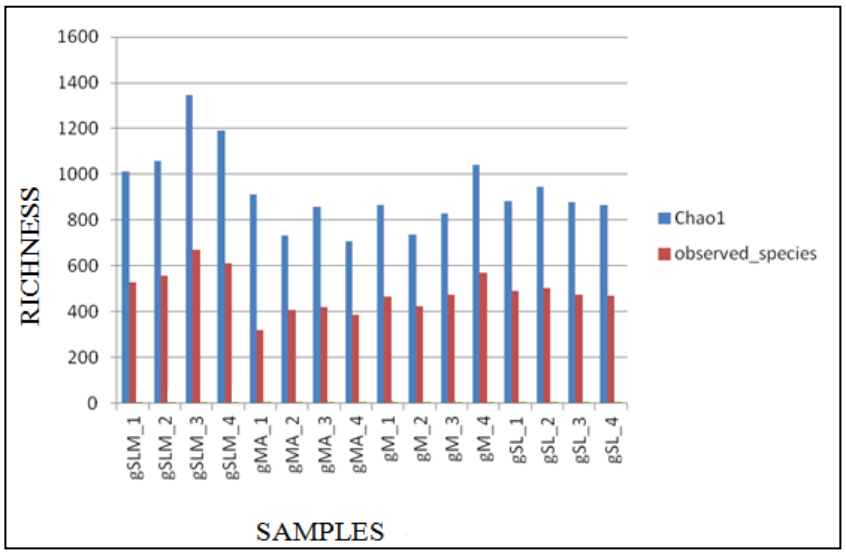

Figure 3a: Bacterial richness for genomic DNA samples

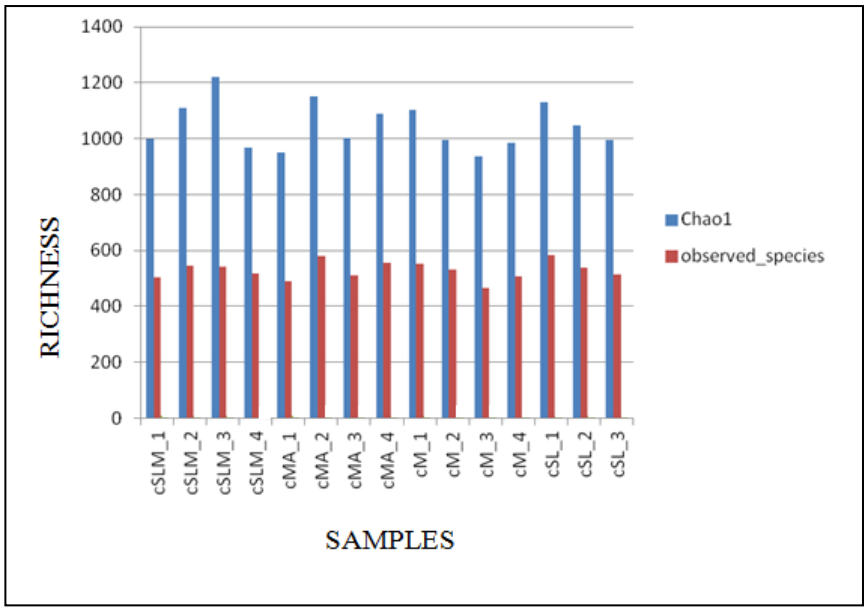

Figure 3b: Bacterial richness for cDNA samples

Comparing these values with those found in other studies is difficult due to the positive associations between sample size and number of OTUs and difference in average sequence length. Nacke et al. (2011) have analyzed German soils with different local management usage and found average lower values of Shannon diversity.

Bacterial assemblages

The 10349 representative OTUs (at similarity threshold of 3%) were compared to known sequence s by BLAST searches against the SILVA database (Quast et al., 2013).

The dominant phyla common to all treatments and replicates were *Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Cyanobacteria, Firmicutes and Verrucomicrobia* (see figure 4). The absolute counts for phylum of bacterial are present in table S2a and S2b for genomic DNA and cDNA (Supplemental material).

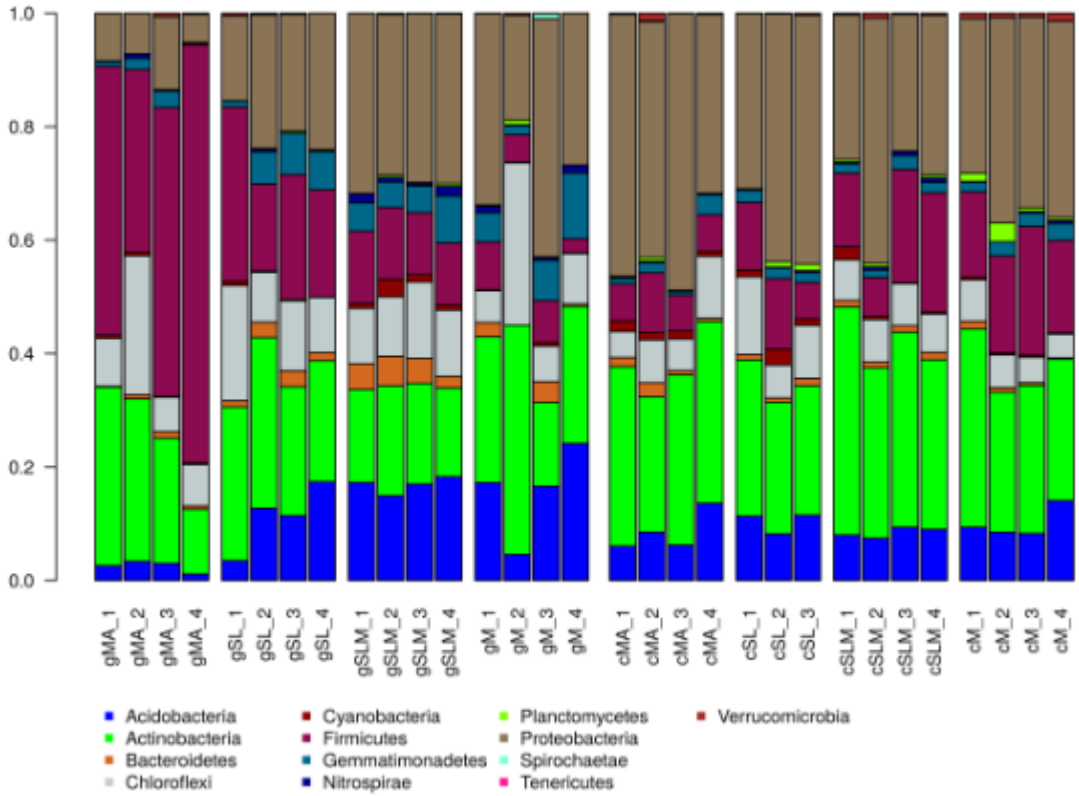

Figure 4: Relative abundance of bacteria of bacteria phyla across samples

Principal component analysis of genomic DNA showed that the treatments were separated along the first two PCA axes that explained 41% and 18% of total variance (see figure 5a for genomic DNA and figure S2a for cDNA).

Firmicutes, Proteobacteria and *Actinobacteria* were the phyla most contributing to sample separation (see figure 5b for genomic DNA and figure S2b for cDNA; the autovector matrix is reported in table S3). The taxonomic composition was significantly different among treatments as determined by ANOSIM analysis ($P<0.05$).

Firmicutes

The average relative abundance of *Firmicutes* across all samples was 17.6%. It was predominant in all genomic MA replicates (average abundance>50%). The relative abundance in the other treatments was 21,2% for slurry, 11,5% for slurry+mineral and only 5,7% for mineral (see figure 4). This trend is in agreement with an increasing C/N trend of fertilization. However, a such association between *Firmicutes* and the C/N fertilizer was not evident for the cDNA samples. The most striking difference was between the relative *Firmicutes* abundances in genomic corresponding cDNA MA samples. In all treatments and replicates, the *Bacilli* was the *Firmicutes* class most represented (>80%), followed by *Clostridia* (>8%) (see figure 6). The absolute counts for classes are reported in table S4a and S4b for genomic DNA and cDNA.

The *Bacilli* were dominated by the *Bacillales* in most samples (see figure 7). The only exception was sample cMA_1 and cM_2 that were rich in *Lactobacillus*. The absolute counts of bacterial orders are present in table S5a and S5b for genomic DNA and cDNA (Supplemental material).

Figure 8 reports the relative abundance of OTUs assigned to the order of *Bacillales*. Members of the *Bacillaceae* family were observed in all samples at high frequency (76 %). The *Alyciclobacillaceae* relative abundance was noticeable because present only in genomic but not in cDNA samples (Figure 8). The absolute counts for family of bacterial are present in table S6a and S6b for genomic DNA and cDNA (Supplemental material).

Proteobacteria

The *Proteobacteria* relative abundance was highly variable among treatments (see figure 4). The relative abundance in cDNA samples was higher than in corresponding genomic samples with *Proteobacteria* representing an average of 41,5% of all bacteria in cDNA samples compared to the 8,2% found in genomic samples. The *Alphaproteobacteria* was the most abundant class across treatments (see figure 9).

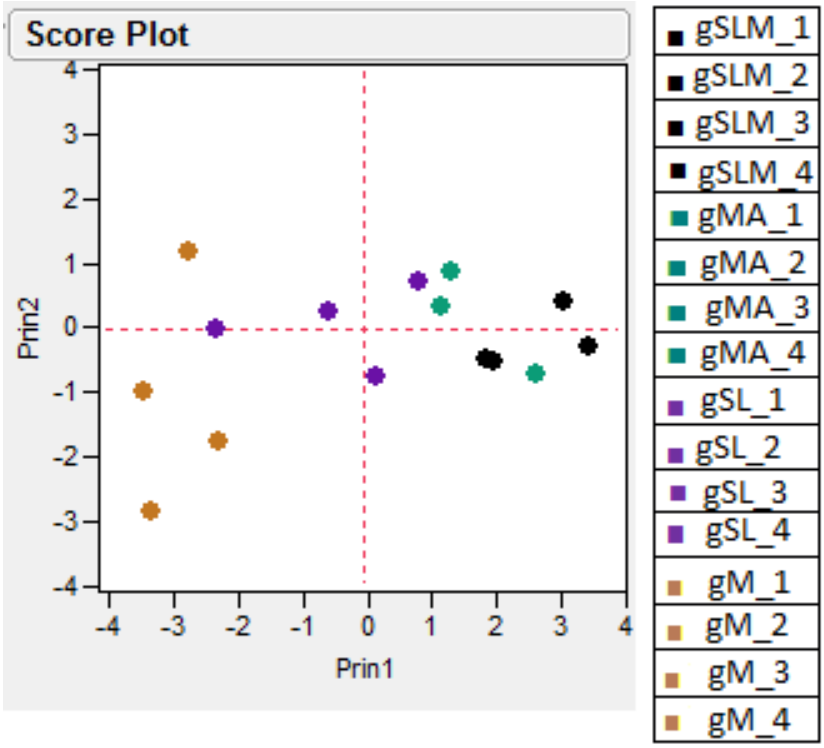

Figure 5a: Principal component analysis of phyum bacteria for genomic DNA

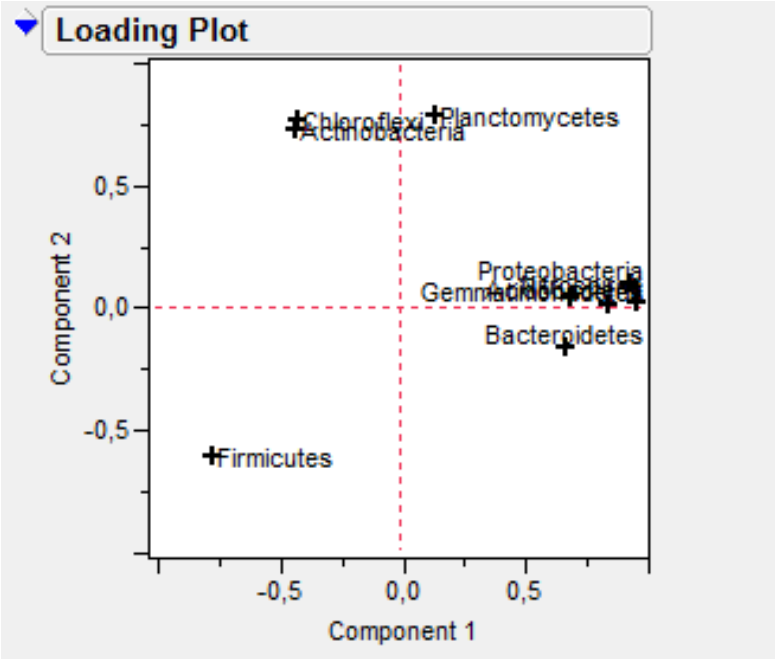

Figure 5b: Principal component analysis of phyum bacteria for cDNA

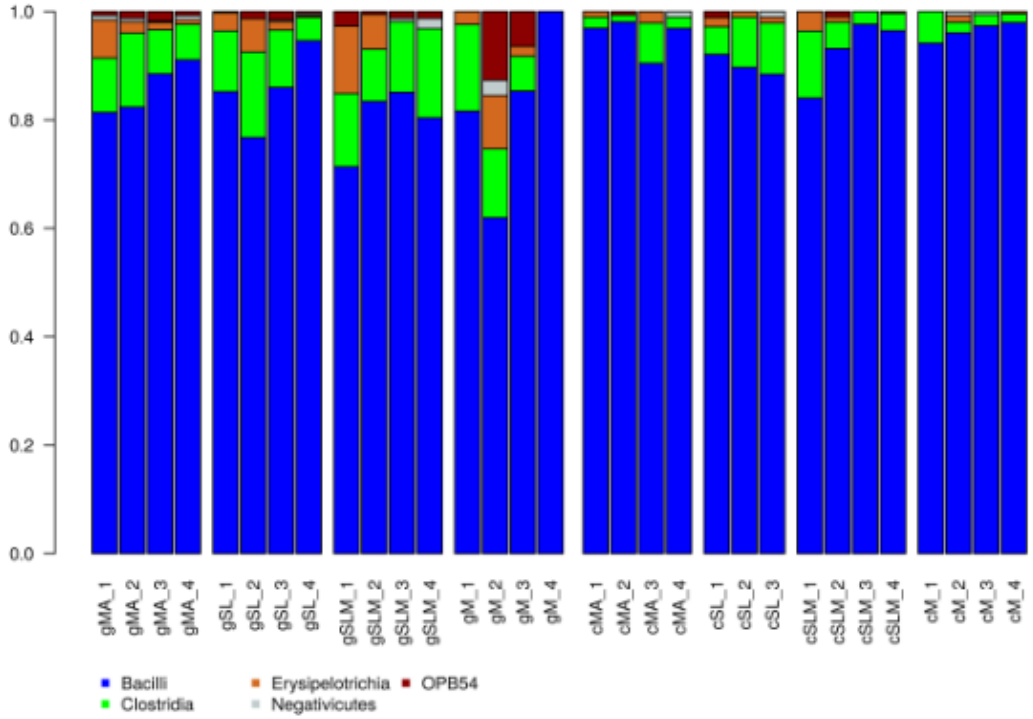

Figure 6: Relative abundance of *Firmicutes* class across samples

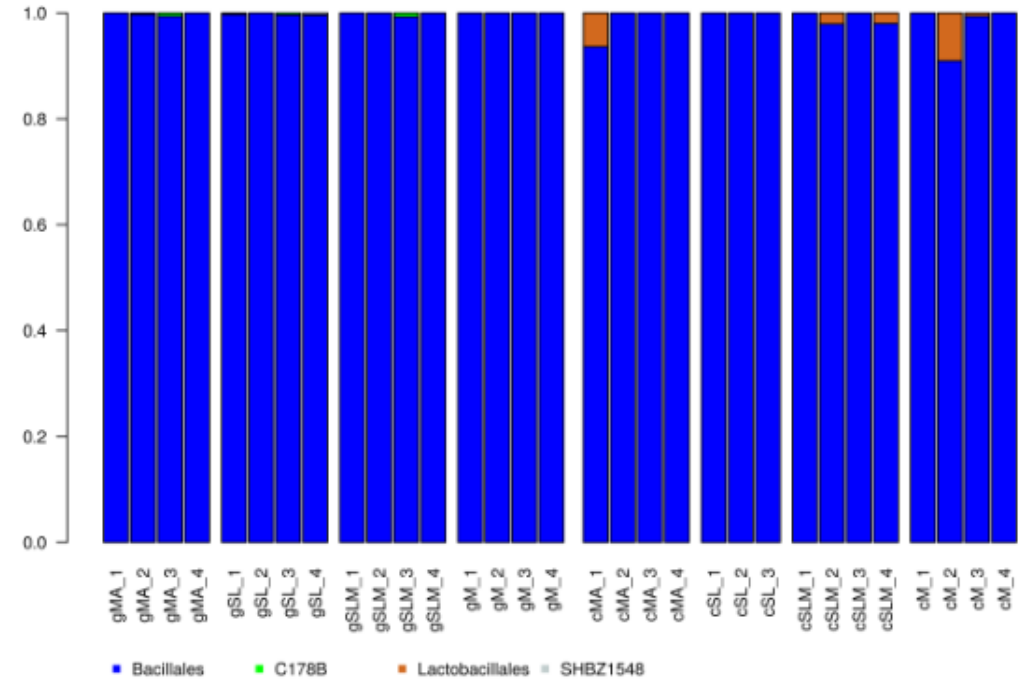

Figure 7: Relative abundance of *Firmicutes* order across samples

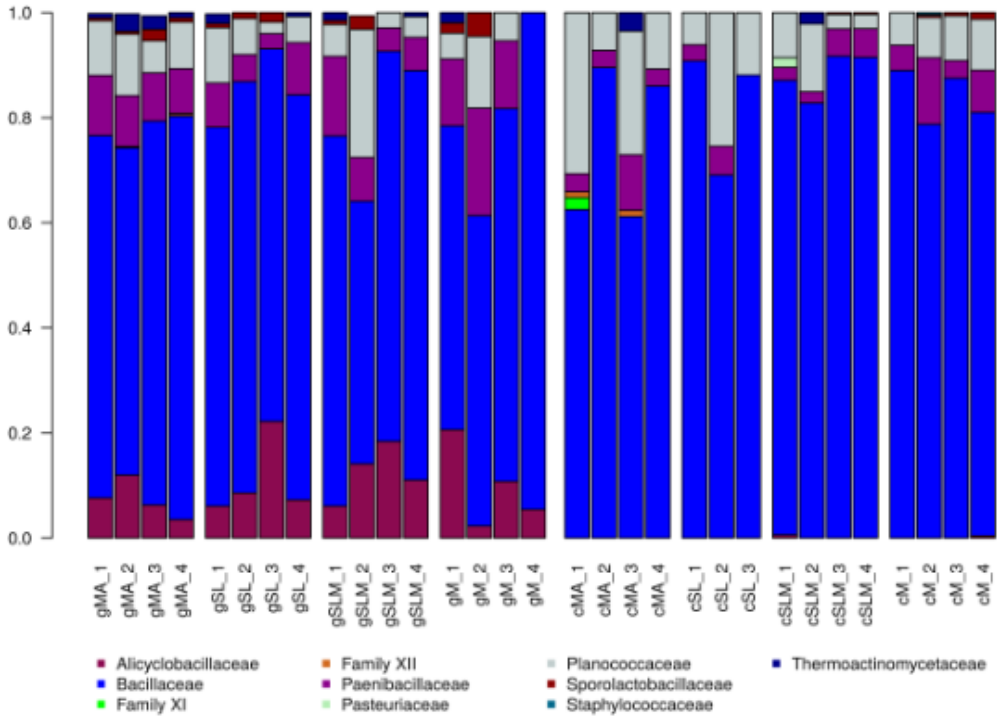

Figure 8: Relative abundance of *Bacillaceae* order across samples

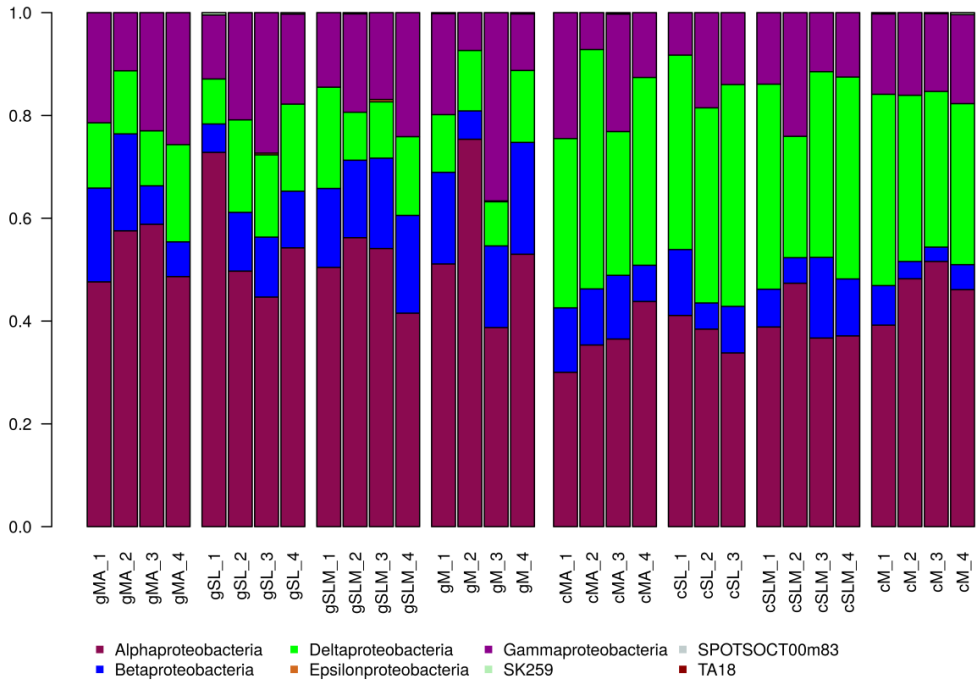

Figure 9: Relative abundance of *Proteobacteria* class across samples

On average most of the variation in relative abundance of *Proteobacteria* classes was noticed between cDNA and genomic samples within, rather than between, treatments. For example the Alphaproteobacteria were more present in genomic samples (>50%) compared to cDNA. The Deltaproteobacteria depicted the opposite pattern being relatively more abundant in cDNA than in the corresponding genomic samples (see figure 9). Within the *Alphaproteobacteria* six orders were identified (see figure 10).

Interestingly the *Alphaproteobacteria* with the highest relative abundance was *Rizhobiales* followed by *Rhodospirillales* and *Sphingomonadales*. Other less represented orders were *Caulobacteriales* and *Rhodobacteriales* (see figure 10).

Acidobacteria

Three classes of the phylum *Acidobacteria* were identified (see figure 11). The most represented class was *Acidobacteria* followed by *Holophagae.* We didi not notice significant differences among samples at class level.

Actinobacteria

Nine classes of *Actinobacteria* were identified (see figure 12), with 2 of these, namely *Actinobacteria* and *Thermoleophila* representing more than 70% of all *Actinobacteria* in studied soils (see figure 13).

Management-sensitive microbial taxa

Next, we focused on the genera that showed significant differences between soils treated with either manure or mineral fertilizer. As expected several *Bacilli* showed higher counts in manure compared to mineral samples. The most significant differences were observed for the genera *Bacillus*, *Paenibacillus*, *Tumebacillus* et al. (see table 3). *Acidobacteria* such as Candidatus *solibacter* and *Blastocatella* were significantly more abundant in mineral than in manure samples.

Taxon (Phylum,Class,Order,Family,Genus)	Manure	Mineral
Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	456.5	191.25
Firmicutes;Bacilli;Bacillales;Alicyclobacillaceae;Tumebacillus	41.5	29.5
Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Paenibacillus	37	11.75
Firmicutes;Bacilli;Bacillales;Planococcaceae;Lysinibacillus	30.75	9.25
Firmicutes;Bacilli;Bacillales;Thermoactinomycetaceae;Planifilum	6.75	1
Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Oxalophagus	6.5	4
Proteobacteria;Gammaproteobacteria;Xanthomonadales;uncultured;uncultured bacterium	3	12.75
Acidobacteria;Acidobacteria;Subgroup 3;Unknown Family;Candidatus Solibacter	1.75	21
Acidobacteria;Acidobacteria;Subgroup 4;Unknown Family;Blastocatella	0.25	17

Table 3: Bacteria genera with different abundance between manure and mineral samples. Only general with a total average count higher than 4 and for which a two sample t test gave a value unassociated probability below 0,05 are listed

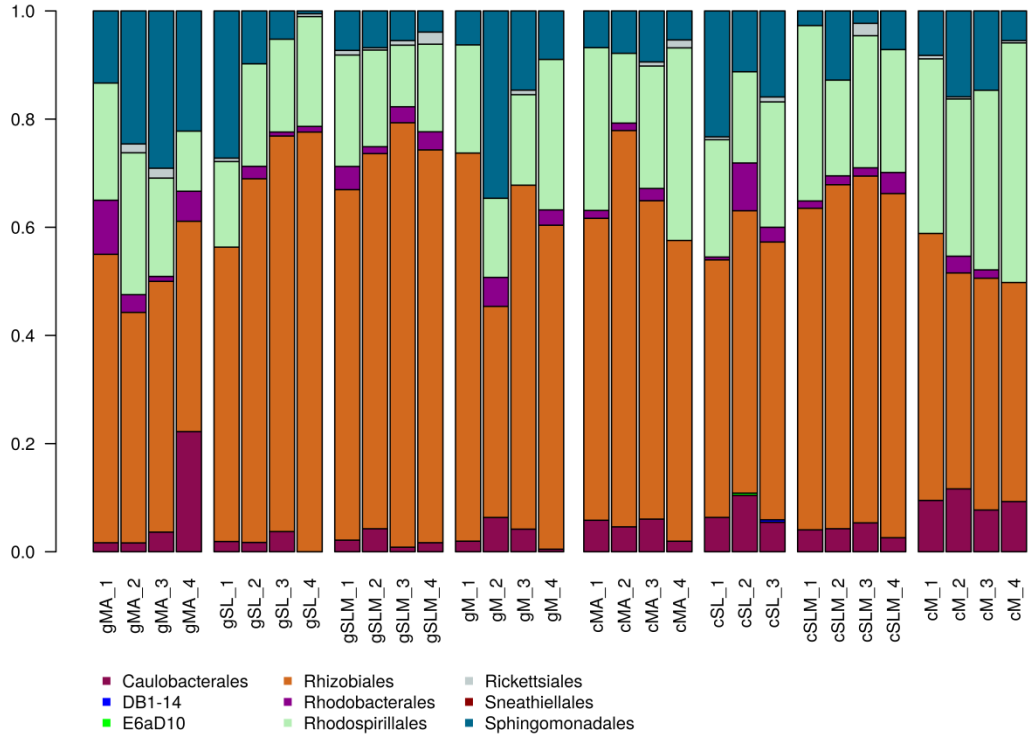

Figure 10: Relative abundance of *Proteobacteria* classes across samples

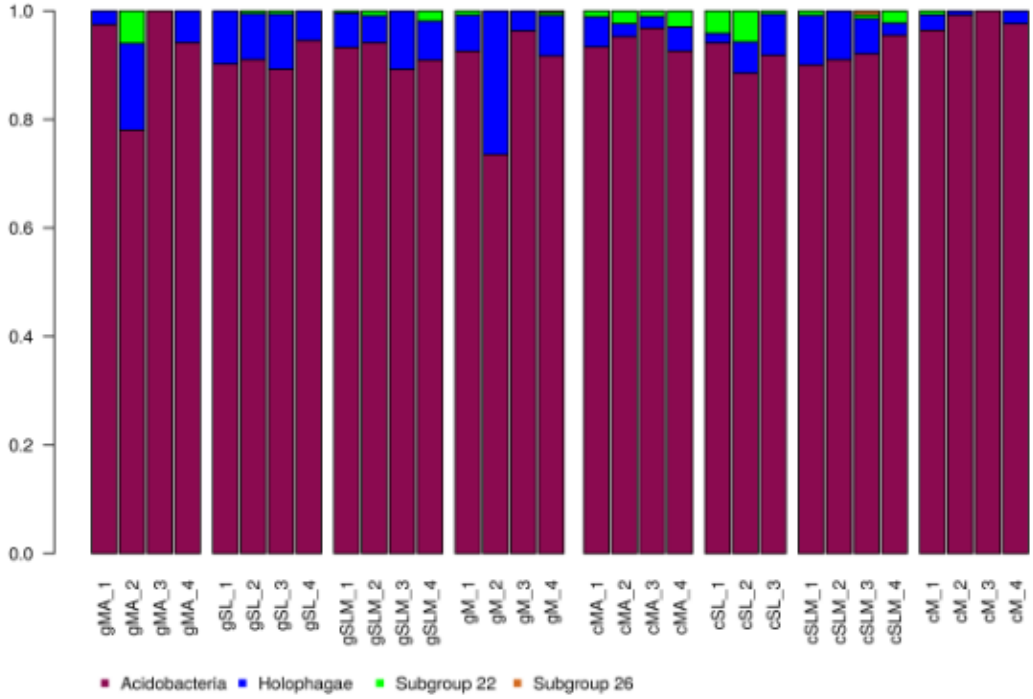

Figure 11: Relative abundance of *Acidobacteria* classes across samples

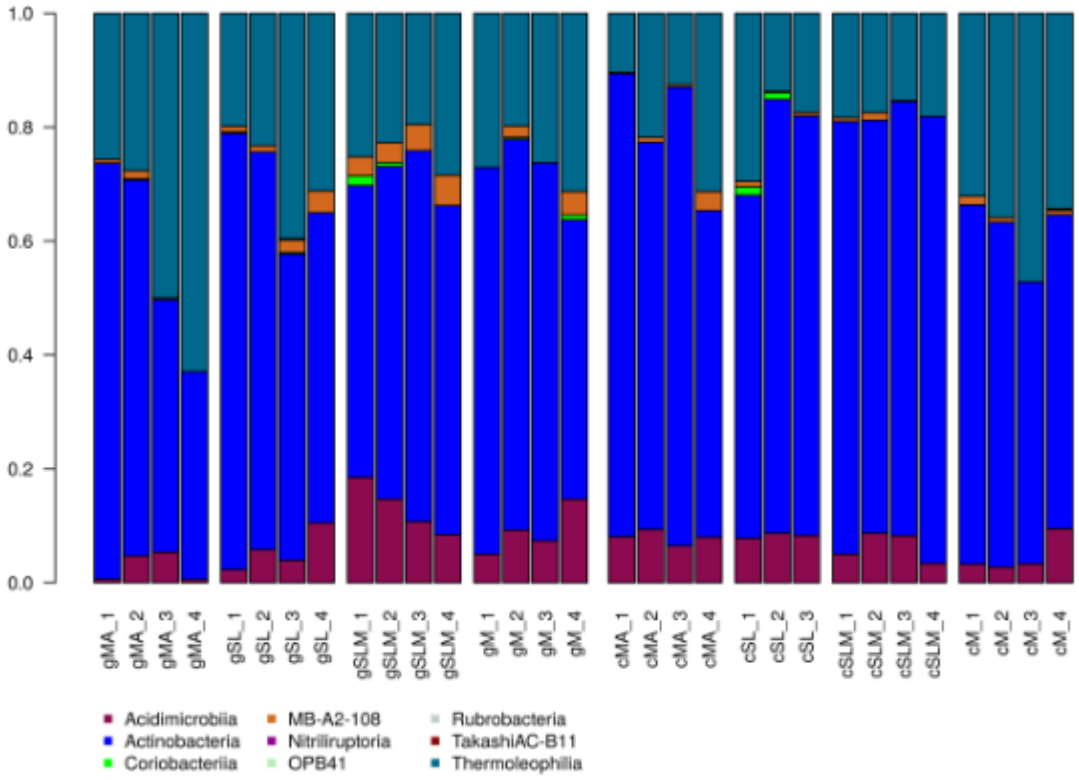

Figure 12: Relative abundance of *Actinobacteria* classes across samples

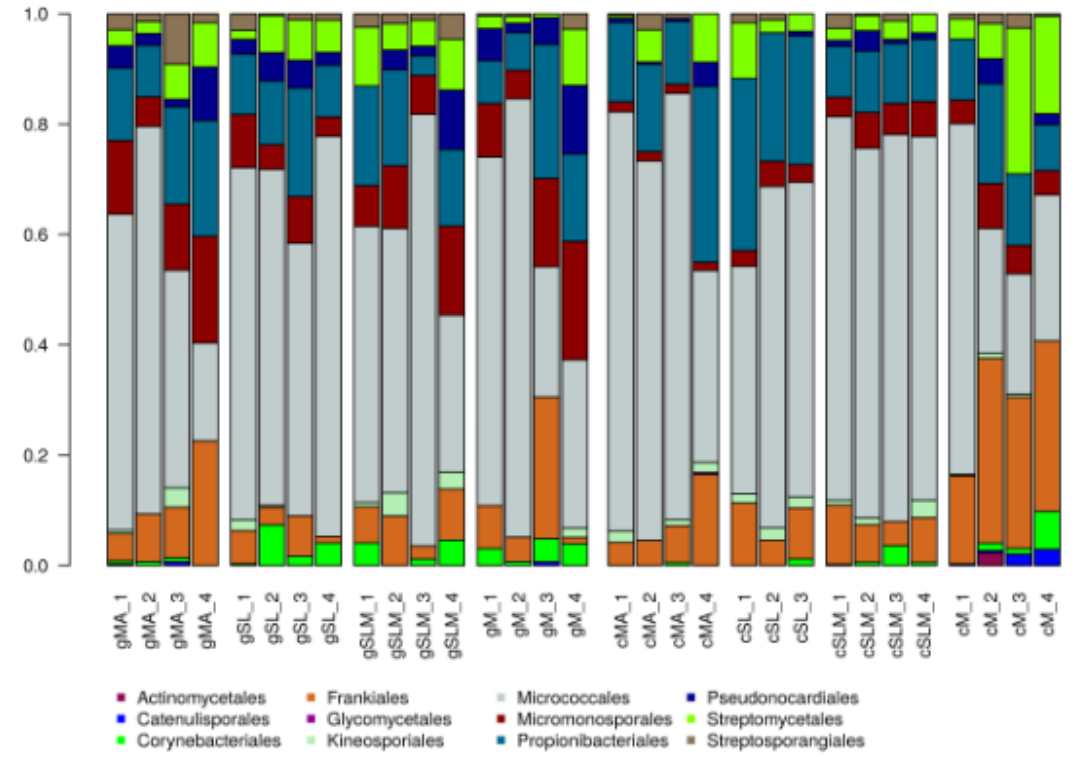

Figure 13: Relative abundance of *Actinobacteria* orders across samples

Discussion

In the present work we have analyzed the bacteria taxonomic compositions of soil manged with short term organic and conventional farming. The organic farming system was represented by a fertilization strategy based on manure and slurry, while the conventional system was based on ENTEC26® a mineral fertilization source. Intermediate level organic/conventional systems were represented by a mix of cattle slurry and mineral. The field trials we investigated did not experience a long history of conventional and organic fertilization and thus the observed effects should be considered as short term responses. We cannot speculate on the persistences of these effects in repeated cycles of conventional or organic fertilization. Accordingly, we cannot rule out the hypothesis that a community structure will evolve by reducing or enhancing some of these differences in response to repeated fertilizations of the same type. The microbial communities assessed from cDNA were substantially differentiated from those assessed from corresponding genomic templates an observation that indicated that many of the sequences detected in genomic samples belong to species that are barely detectable in cDNA-templates. Technical or biological reasons could be implicated. Some bacteria species could be hardly tractable for RNA extractions or retrotranscriptions. Alternatively, one could think that the different cDNA relative abundances simply reflect the different growth rate of analyzed taxa. Indeed the observation that many OTUs showing a such behaviour belong to the Firmicutes and that this phylum is rich of spore forming genera, gives support to the hypothesis.

Mutivariate analysis of the effects of farming systems detected significant differences though the high variability within treatments (both in cDNA and genomic samples). Again this could be intrinsic to the "short term nature" of our observations or linked to variability present in fertilizers or pre-existing in the field. A larger experimental design, with dedicated controls will surely solve some of these questions.

The *Firmicutes* were clearly more abundant in the organic tratment samples. Many genera of this phylum have been found during meso and thermophilic degradation processes of organic matter such as manure or compost (Ryckeboer et al., 2003) and are capable of degrading complex organic materials (Watanabe et al., 2007, Charbonneau et al., 2012). Several *Bacilli* and *Paenibacilli* were more represented in manure than mineral samples. Kuramae et al. (2010) reported high relative abundance of *Firmicutes* in nutrient rich soils of the Netehrlands. More interestingly Hartman et al. (2010) have reported that the abundance of several *Firmicutes* genera is correlated positively with soil treated with manure and slurry fertilization for many years. Unfortunately, we did not measure the *Firmicutes* content of manure or slurry administered to soils and thus the hypothesis that the *Firmicutes* increase is substantially an amendant effect cannot be ruled out. The convergence of

results obtained from short term (our study) and long term experiments (Hartman et al., 2015, Kuramae et al., 2010), however, suggest that the *Firmicutes* is a management-sensitive microbial taxa whose abundance could be used as predictor of soil potential for degrading complex organic molecules. Another interesting observation was the relative high content of *Acidobacteria* in mineral samples compared to manure samples. Fierer et al. (2007) have proposed that *Acidobacteria* generally prefer soil enviroments of low resources and acidic pH. Indeed, Hartman et al. (2015) found that the genus *Candidatus solibacter* was associated with systems not receiving organic matter. Members of this genus have been suggested to be slow growing, oligotroph adapted to nutrients poor limited enviroments (Ward et al., 2009). Indeed we found that this genus was more abundant in mineral compared to manure samples. Finally we would like to mention that a possible drawback of organic fertilization is associated to the delivery of bacteria pathogenic for humans or animals along with manure. Another possible unitended effect of organic fertilization may derive from the presence of antibiotics in feces or urine of animals. Udikovic-Kolic et al, (2014) have indicated that soil fertilized with manure have significantly higher counts of antibiotic resistant species than soil managed with conventional antibiotic. Our list of management sensitive-taxa did not reveal potentially pathogenic bacteria. However we warn against over-interpreting these evidences as the sequencing depth of our experiments could have inadequate resoultion power.

Acnowledgments

We wish to acknowledge the collaboration of Prof. Roggero, dr Seddaiu; dr Roberto Lai and dr. Antonio Pulina for sharing information on soil samples and insightful for discussion.

We also wish to thanks Prof. Daniel and dr. Wemheuer for the time spent in the their laboratory in Germany.

References

Bruchem J.V., Schiere H., and Keulen H.V., 1999. Dairy farming in the Netherlands in transition towards more efficient nutrient use. Livestock production science 61: 2-3. ISSN 0301-6226-p 145-153.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Pena A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature methods 7.5:335-36.

Charbonneau D.M., Meddeb-Mouelhi F., Boissinot M., Sirois M., Beauregard M., 2012. Identification of thermophilic bacterial strains producing thermotolerant hydrolytic enzymes from manure compost. Indian Journal of microbiology 52(1): 41-47.

Demurtas C.E., 2013. Sustainable management of nitrogen fertilization in irrigated forage systems in nitrate vulnerable zones. PhD Thesys. PhD School in science and biotechnology of agricultural and forestry system of food production, University of Sassari,IT.

Fierer N., Breitbart M., Nulton J., Salomon P., Lozupone C., Jones R., Robeson M., Edwards R.A., Felts B., Rayhawk S., Knight R., Rohwer F., Jackson R.B., 2007. Metagenomic and Small-Subunit rRNA Analyses Reveal the Genetic Diversity of Bacteria, Archaea, Fungi, and Viruses in Soil. Applied and environmental microbiology, 73.21: 7059-66.

Hartmann M., Howes C.G., Abarenkov K., Mohn W.W., Nilsson R.H., 2010. V-Xtractor: an open-source, high-throughput software tool to identify and extract hypervariable regions of small subunit (16S/18S) ribosomal RNA gene sequences. Journal of Microbiology Methods 83: 250-253.

Hartmann M., Frey B., Mayer J., Mäder P., Widmer F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. The ISME journal 9:1177-1194.

Hu J., Wang J., Dai J., Chen R., Zhang J., Wong M.H., 2011. Microbial functional diversity, metabolic quotient and invertase activity of a sandy loam soil as affected by long-term application of organic amendment and mineral fertilizer. Journal of Soils and Sediments 11:271-280.

Janssen P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and environmental microbiology 72.3: 1719-28.

Kuramae E.E., Gamper H.A., Yergeau E., Piceno Y.M., Brodie E.L., DeSantis T.Z., Andersen G.L., van Veen J.A., Kowalchuk G.A., 2010. Microbial secondary succession in a chronosequence of chalk grasslands. The ISME Journal 4:711-715.

Nacke H., Thürmer A., Wollherr A., Will C., Hodac L., Herold N., Schöning I., Schrumpf M., Daniel R., 2011. Pyrosequencing-Based Assessment of Bacterial Community Structure Along Different Management Types in German Forest and Grassland Soils. PLOS ONE 6.2: e17000.

Quast C., Pruess E., Yilmaz P., Gerken J., Schweer T., Peplies J., Glöcner F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research (DI): D590-6

Ryckeboer J., Mergaert J., Coosemans J., Deprins K., Swings J., 2003. Microbiological aspects of biowaste during composting in a monitored compost bin. Journal Applied Microbiology, 94(1): 127-37.

Shange R.S., Ankumah R.O., Ibekwe A.M., Zabawa R., Dowd S.E., 2012. Distinct Soil Bacterial Communities Revealed under a Diversely Managed Agroecosystem. PLOS ONE 7 (7): e40338.

Siala A., and Gray T.R., 1973. Growth of *Bacillus subtilis* and spore germination in soil observed by a fluorescent-antibody technique. Journal of General Microbiology 8: 191-198.

Udikovic-Kolic N., Wichmann F., Broderick N.A., Handelsman J., 2014. Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. PNAS 111(42): 15202-15207.

Ward N.L., Challacombe J.F., Janssen P.H., Henrissat B., Coutinho P.M., Wu M., Xie G., Haft D.H., Sait M., Badger J., Barabote R.D., Bradley B., Brettin T.S., Brinkac L.M., Bruce D., Creasy T., Daugherty S.C., Davisen T.M., DeBoy R.T., Detter J.C., Dodson R., Durkin a.S., Ganapathy A., Gwinn-Giglio M., Han C.S., Khouri H., Kiss H., Kothari S.P., Madupu R., Nelson K.E., Nelson W.C., Paulsen I., Penn K., Ren Q., Rosovitz M.J., Selengut J.D., Shrivastava S., Sullivan S.A., Tapia R., Thompson L.S., Watkins K.L., Yang Q., Yu C., Zafar N., Zhou L., Kuske C.R., 2009. Three genomes from the phylum *Acidobacteria* provide insight into the lifestyles of these microorganism in soils. Applied Environmental Microbiology, 75: 2046-2056.

Watanabe K., Nagao N., Yamamoto S.,Toda T., Kurosawa N., 2007. *Thermobacillus composti* sp. Nov. a moderately thermophilic bacterium isolated from a composting reactor. International Journal Systematic Evolutionary Microbialogy, 57: 1473-1477.

Supplemental material

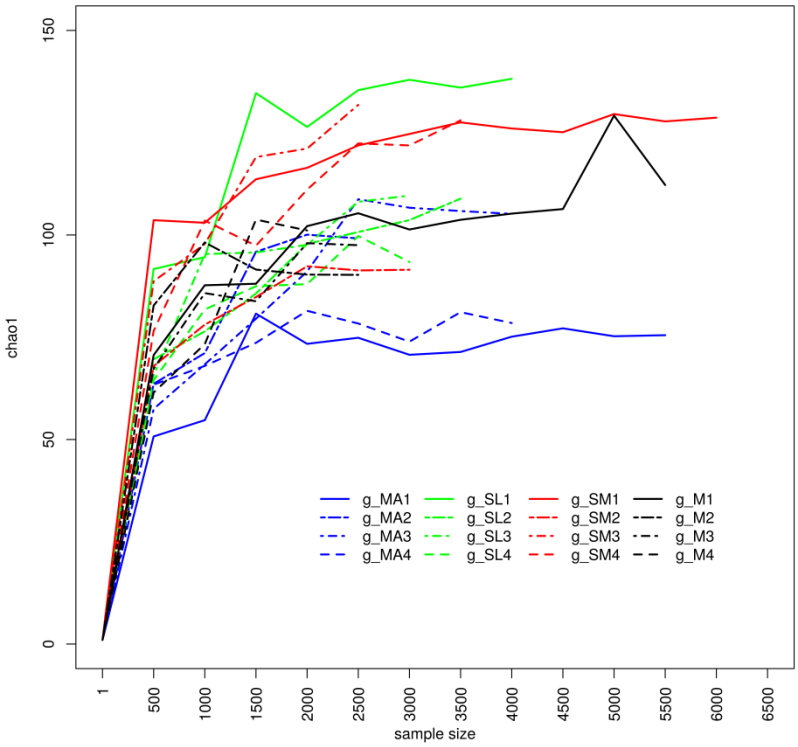

Figure S1a: Rarefaction curve (chao1) at a sequence similarity level of 20%

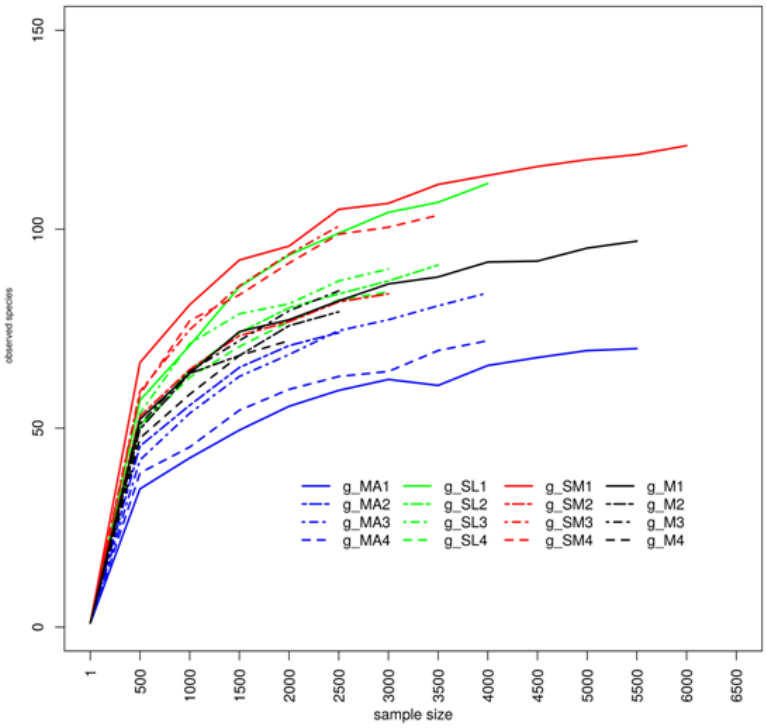

Figure S1b: Rarefaction curve (observed species) at a sequence similarity level of 20%

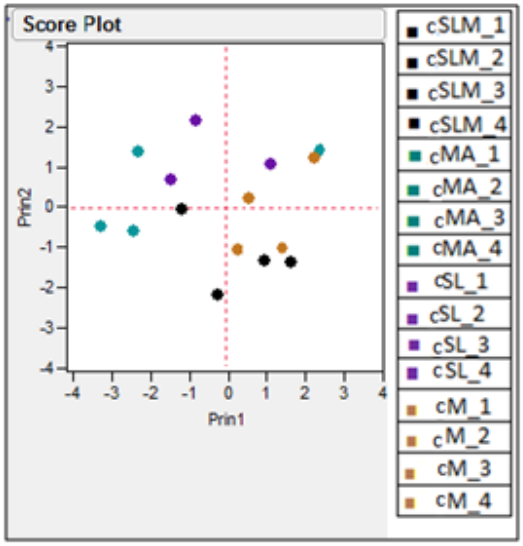

Figure S2a: Principal component analysis of phyum bacteria for cDNA

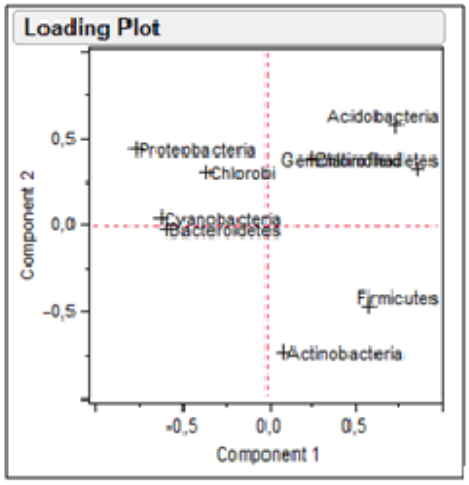

Figure S2b:Principal component analysis of phyum bacteria for cDNA

Genomic DNA	Shannon index	cDNA	Shannon index
gSLM_1	8,047,438,602	**cSLM_1**	7,487,690,145
gSLM_2	8,305,369,008	**cSLM_2**	7,696,653,172
gSLM_3	8,791,758,791	**cSLM_3**	7,627,075,371
gSLM_4	8,586,193,801	**cSLM_4**	784,879,679
gMA_1	5,956,719,387	**cMA_1**	7,540,252,935
gMA_2	7,311,353,341	**cMA_2**	8,276,311,093
gMA_3	6,965,786,156	**cMA_3**	7,540,837,111
gMA_4	6,779,791,903	**cMA_4**	808,728,345
gM_1	8,023,929,385	**cM_1**	7,961,226,045
gM_2	7,362,346,013	**cM_2**	8,118,167,763
gM_3	802,429,366	**cM_3**	7,627,803,059
gM_4	826,557,674	**cM_4**	8,070,004,345
gSL_1	7,952,020,269	**cSL_1**	8,307,012,149
gSL_2	7,926,338,332	**cSL_2**	8,085,528,698
gSL_3	7,772,309,111	**cSL_3**	8,071,034,121
gSL_4	755,085,632		

Table S1a: Bacterial richness with Shannon index at 97% of similarità

Genomic DNA	Shannon index	cDNA	Shannon index
gSLM_1	4.821022	**cSLM_1**	3.84669
gSLM_2	4.755872	**cSLM_2**	3.769723
gSLM_3	4.695733	**cSLM_3**	3.688878
gSLM_4	4.52394	**cSLM_4**	3.871612
gMA_1	3.18475	**cMA_1**	3.763822
gMA_2	2.915311	**cMA_2**	4.143191
gMA_3	3.839484	**cMA_3**	4.199414
gMA_4	2.198126	**cMA_4**	3.673426
gM_1	4.211068	**cM_1**	3.770351
gM_2	4.309134	**cM_2**	4.025851
gM_3	3.932829	**cM_3**	3.631368
gM_4	4.437044	**cM_4**	3.882328
gSL_1	4.55368	**cSL_1**	4.219094
gSL_2	4.18705	**cSL_2**	4.156919
gSL_3	4.232995	**cSL_3**	4.06336
gSL_4	4.04607		

Table S1b: Bacterial richness with Shannon index at 80% of similarity

Taxon	gSLM_1	gSLM_2	gSLM_3	gSLM_4	gM_1	gM_2	gM_3	gM_4	gMA_1	gMA_2	gMA_3	gMA_4	gSL_1	gSL_2	gSL_3	gSL_4
Acidobacteria;Acidobacteria	223	208	234	240	331	90	50	235	16	38	44	39	149	172	242	46
Acidobacteria;Holophagae	27	11	16	19	27	2	18	9	1	1	0	8	17	16	14	5
Actinbacteria;Acidimicrobiia	28	42	44	19	53	10	55	16	1	3	17	20	13	26	33	9
Actinbacteria;Actinbacteria	170	167	122	130	177	231	409	144	62	344	142	279	178	312	171	301
Actinbacteria;MB-A2-108	12	10	8	12	15	8	12	0	0	3	1	6	7	5	12	4
Actinbacteria;Thermoleophilia	51	65	60	64	113	175	118	57	107	121	160	117	131	104	98	78
Bacteroidetes;Cytophagia	42	11	20	1	0	2	0	13	1	2	0	7	5	4	7	5
Bacteroidetes;Flavobacteriia	3	13	11	5	0	0	0	2	0	0	0	2	0	11	0	2
Bacteroidetes;Sphingobacteriia	20	53	27	22	6	19	0	38	8	0	16	2	15	23	14	9
Candidate division WS3;uncultured	4	1	8	9	2	1	0	1	1	0	0	0	1	1	1	0
Chlorobi;Ignavi	6	2	11	7	0	2	0	6	1	1	5	0	7	1	2	5
Chloroflexi;Anaerolineae	67	18	37	46	22	10	103	9	7	11	21	42	12	22	32	74
Chloroflexi;Ardenticatenia	0	4	3	1	0	1	2	0	0	0	0	0	0	0	0	14
Chloroflexi;Caldilineae	0	1	2	7	0	2	10	0	2	0	0	3	0	0	0	16
Chloroflexi;Chloroflexia	18	40	23	29	14	40	53	7	1	28	7	44	20	20	16	52
Chloroflexi;Elev-1554	2	0	0	0	1	0	0	0	0	1	0	4	1	0	0	0
Chloroflexi;Gitt-GS-136	0	14	4	7	9	12	32	1	1	7	1	40	0	1	2	20
Chloroflexi;JG30-KF-CM66	8	2	14	10	13	2	10	1	0	1	0	5	3	4	11	1
Chloroflexi;KD4-96	25	21	16	15	18	42	72	9	18	20	5	72	32	11	24	21
Chloroflexi;Ktedono	7	26	9	19	23	81	22	39	31	29	30	40	51	55	25	28
Chloroflexi;S085	30	3	4	1	4	4	12	0	4	1	0	20	14	3	5	11
Chloroflexi;TK10	11	9	4	5	13	14	14	17	9	10	4	18	3	4	4	9
Chloroflexi;Thermomicrobia	7	11	15	15	11	39	63	5	17	18	14	50	24	11	10	39
Chloroflexi;uncultured	18	6	8	10	2	6	26	1	5	1	4	21	12	0	6	7
Cyano;Cyano	18	38	8	0	1	11	2	4	5	7	1	8	1	0	1	10
Firmicutes;Bacilli	137	156	132	127	37	302	44	93	1000	577	654	392	276	175	263	380
Firmicutes;Clostridia	21	18	25	26	0	21	9	7	72	71	60	65	34	36	12	50
Firmicutes;Erysipelotrichia	0	12	23	0	0	3	7	2	9	50	10	10	5	14	1	15
Gemmatimonadetes;Gemmatimonadetes	69	66	72	119	173	48	23	104	4	12	42	29	106	84	99	15
Nitrospirae;Nitrospira	6	12	23	25	20	5	3	7	2	2	3	12	1	7	4	0
Planctomycetes;Planctomycetacia	2	7	1	6	1	0	4	1	0	0	0	0	2	2	1	1
Proteobacteria;Alphaproteobacteria	237	235	233	179	212	114	205	239	36	60	110	61	134	174	192	158
Proteobacteria;Betaproteobacteria	77	63	71	82	87	34	15	98	5	23	14	20	35	40	39	12
Proteobacteria;Deltaproteobacteria	48	39	91	66	56	29	32	53	14	16	20	13	48	63	60	19
Proteobacteria;Gammaproteobacteria	74	80	67	104	44	101	20	226	19	27	43	12	82	73	62	27
Spirochaetae;Spirochaetes	1	0	0	0	0	4	0	13	1	0	0	0	2	2	0	0

Table S2a: Absolute counts of OTUs assigned to bacteria phyla

Taxon	cSLM_1	cSLM_2	cSLM_3	cSLM_4	cM_1	cM_2	cM_3	cM_4	cMA_1	cMA_2	cMA_3	cMA_4	cSL_1	cSL_2	cSL_3
Acidobacteria;Acidobacteria	128	127	101	108	134	125	124	204	84	187	120	90	157	108	159
Acidobacteria;Holophagae	9	3	10	11	4	1	0	5	5	9	3	2	13	7	3
Actinobacteria;Acidimicrobiia	42	15	39	30	17	10	13	35	38	38	33	29	28	30	32
Actinobacteria;Actinobacteria	388	346	324	456	326	221	193	204	382	273	240	360	249	262	247
Actinobacteria;MB-A2-108	1	0	6	4	8	3	0	3	1	16	3	2	2	1	4
Actinobacteria;Thermoleophilia	78	80	78	110	166	131	184	127	49	149	77	56	59	47	121
Bacteroidetes;Cytophagia	1	2	2	3	0	0	0	0	3	0	2	1	3	1	1
Bacteroidetes;Flavobacteriia	0	0	2	0	1	0	0	0	5	0	1	0	0	1	0
Bacteroidetes;Sphingobacteriia	16	15	11	14	19	11	7	3	16	8	32	10	14	9	13
Candidate division WS3;uncultured Latesci	1	3	0	1	0	0	0	0	0	6	2	0	3	0	0
Candidate division WS3;uncultured	9	2	2	0	2	0	0	0	3	2	3	0	0	4	3
Chlorobi;Ignavi	1	0	0	1	2	2	0	6	7	0	2	0	4	1	1
Chloroflexi;Anaerolineae	27	24	30	22	16	9	2	2	29	22	33	23	31	18	28
Chloroflexi;Chloroflexia	22	12	16	10	18	0	6	3	8	28	18	5	14	13	52
Chloroflexi;Elev-1554	2	0	1	1	1	0	3	2	0	1	0	0	0	0	0
Chloroflexi;Gitt-GS-136	8	2	4	10	3	6	2	1	3	8	1	7	13	7	8
Chloroflexi;JG30-KF-CM66	5	5	7	6	7	5	7	7	6	2	4	3	7	4	17
Chloroflexi;KD4-96	13	23	26	15	11	10	16	19	10	24	24	19	32	21	27
Chloroflexi;Ktedono	8	5	7	15	17	21	9	6	1	34	9	1	16	1	15
Chloroflexi;S085	1	4	5	5	9	1	3	4	1	13	8	7	11	2	10
Chloroflexi;SHA-26	0	1	0	1	6	7	2	4	0	0	0	0	0	0	0
Chloroflexi;TK10	10	12	5	8	11	10	9	6	7	18	10	4	7	8	13
Chloroflexi;Thermomicrobia	8	3	8	12	6	13	8	6	1	7	2	10	5	7	31
Chloroflexi;uncultured	2	2	1	1	2	3	0	0	0	2	0	3	0	0	1
Chloroflexi;uncultured Bellilinea sp.	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1
Cyano;Cyano	0	2	5	27	3	3	1	1	26	6	12	16	13	29	11
Firmicutes;Bacilli	289	299	95	163	209	243	330	237	94	93	153	85	84	165	163
Firmicutes;Clostridia	7	10	5	24	13	5	7	4	2	2	2	7	9	17	9
Firmicutes;Erysipelotrichia	0	1	1	7	0	3	0	1	1	0	0	2	1	2	3
Gemmatimonadetes;Gemmatimonadetes	36	27	20	25	25	37	36	44	14	52	26	9	26	28	32
Nitrospirae;Nitrospira	11	12	8	4	2	0	4	8	2	4	4	0	5	2	3
Planctomycetes;Planctomycetacia	0	8	9	7	17	47	10	7	3	0	10	2	11	14	0
Proteobacteria;Alphaproteobacteria	131	154	305	148	158	258	259	237	206	205	217	265	220	249	189
Proteobacteria;Betaproteobacteria	56	46	32	28	31	18	14	25	86	33	67	90	59	33	59
Proteobacteria;Deltaproteobacteria	129	163	152	152	150	173	152	161	226	171	286	203	281	246	174
Proteobacteria;Gammaproteobacteria	41	52	155	53	63	86	76	89	168	59	44	166	91	120	38
Spirochaetae;Spirochaetes	0	0	0	0	0	0	0	0	2	0	2	1	0	0	0

Table S2b: Absolute counts of OTUs in cDNA samples assigned to bacteria phyla

Eigenvectors												
Bacteria;Acidobacteria	0,37415	-0,05855	-0,18614	0,44135	0,01401	0,06200	-0,36014	-0,13772	0,25408	-0,43535	-0,41449	0,22034
Bacteria;Actinobacteria	0,09861	0,33987	-0,02215	-0,49394	0,40384	-0,06862	-0,43194	0,29235	0,07646	0,02869	0,04968	0,42583
Bacteria;Bacteroidetes	-0,21332	0,31722	0,53697	0,14067	0,17862	-0,06381	0,05066	-0,21237	-0,03899	0,29621	-0,60737	0,06272
Bacteria;Candidate division WS3	0,22830	0,43322	0,24576	0,17206	-0,13583	0,22928	0,35116	0,57279	-0,16750	-0,34582	0,00973	-0,00531
Bacteria;Chlorobi	-0,16939	-0,07898	0,54418	0,35682	0,10897	0,16022	-0,44074	-0,07587	-0,05049	-0,11718	0,53580	0,02642
Bacteria;Chloroflexi	0,16667	0,35272	-0,20809	0,36675	0,36671	-0,37966	0,35318	-0,29812	-0,01059	0,06780	0,36285	0,20346
Bacteria;Cyanobacteria	-0,32631	0,24999	-0,23678	0,00848	0,13244	0,72522	0,15363	-0,17383	0,41051	0,03285	0,06960	0,05596
Bacteria;Firmicutes	0,30000	-0,30528	0,28334	-0,32185	0,01682	0,26363	0,32845	-0,37971	-0,21742	-0,17167	0,02869	0,48324
Bacteria;Gemmatimonadetes	0,42443	-0,18888	-0,09589	0,27652	0,11007	0,34032	-0,07783	0,23247	-0,18101	0,68791	-0,00559	0,06693
Bacteria;Nitrospirae	0,34818	0,16912	0,27779	-0,10925	-0,48980	-0,13212	0,00959	-0,08692	0,63300	0,25313	0,17366	0,03377
Bacteria;Planctomycetes	-0,11438	-0,48903	0,18571	0,09021	0,41624	-0,15115	0,31762	0,39342	0,49237	-0,04190	-0,04632	0,08388
Bacteria;Proteobacteria	-0,43041	-0,05458	-0,13959	0,22321	-0,44374	-0,11585	0,01841	0,19226	-0,07542	0,11994	0,00142	0,68902

Table S3: Autovectors of principal component analysis

Taxon	gSLM_1	gSLM_2	gSLM_3	gSLM_4	gM_1	gM_2	gM_3	gM_4	gMA_1	gMA_4	gMA_3	gMA_2	gSL_1	gSL_2	gSL_3	gSL_4
Acidobacteria;Acidobacteria;Acidobacteriales	19	26	24	21	88	31	5	82	5	3	17	5	41	22	32	7
Acidobacteria;Acidobacteria;Subgroup 17	4	2	12	16	2	1	1	0	0	1	0	0	0	0	1	0
Acidobacteria;Acidobacteria;Subgroup 2	2	0	0	1	4	5	0	0	1	0	8	0	0	1	1	0
Acidobacteria;Acidobacteria;Subgroup 3	60	80	40	44	96	22	16	98	3	2	6	14	53	27	53	7
Acidobacteria;Acidobacteria;Subgroup 4	19	23	36	47	26	10	9	8	2	3	0	0	25	54	41	6
Acidobacteria;Acidobacteria;Subgroup 6	110	72	109	100	108	18	18	43	4	23	9	18	24	57	112	23
Acidobacteria;Holophagae;Subgroup 10	11	6	10	4	8	1	18	3	0	7	0	1	2	4	4	5
Acidobacteria;Holophagae;Subgroup 7	16	5	6	15	19	1	0	2	1	1	0	0	13	12	10	0
Actinobacteria;Acidimicrobiia;Acidimicrobiales	28	42	44	19	53	10	55	16	1	20	17	3	13	26	33	9
Actinobacteria;Actinobacteria;Coryneles	2	0	5	6	7	8	3	6	0	2	1	2	3	23	7	1
Actinobacteria;Actinobacteria;Frankiales	4	15	8	12	2	27	18	37	14	24	13	17	13	10	2	18
Actinobacteria;Actinobacteria;Micrococcales	133	80	61	37	54	114	325	34	11	196	56	197	88	190	124	192
Actinobacteria;Actinobacteria;Micromonosporales	12	19	9	21	38	23	21	23	12	15	17	46	15	14	6	29
Actinobacteria;Actinobacteria;Pseudonocardiales	3	6	0	14	22	7	7	7	6	6	2	14	9	16	4	8
Actinobacteria;Actinobacteria;Streptomycetales	8	8	13	12	18	9	5	1	5	6	9	10	13	21	10	5
Actinobacteria;Actinobacteria;Streptosporangiales	2	3	3	6	5	8	2	0	1	4	13	10	2	1	2	9
Actinobacteria;MB-A2-108;actinobacterium	9	7	5	2	3	8	9	0	0	6	0	0	4	0	4	2
Actinobacteria;Thermoleophilia;Gaiellales	37	37	34	31	99	153	84	49	101	80	139	106	88	61	66	57
Actinobacteria;Thermoleophilia;Solirubrobacterales	14	28	26	33	14	22	34	8	6	37	21	15	43	43	32	21
Bacteroidetes;Cytophagia;Cytophagales	39	10	19	1	0	2	0	13	1	6	0	0	5	4	7	5
Bacteroidetes;Sphingobacteriia;Sphingoles	20	53	27	22	6	19	0	38	8	2	16	0	15	23	14	9
Chlorobi;Ignavi;Ignaviles	6	2	11	7	0	2	0	6	1	0	5	1	7	1	2	5
Chloroflexi;Anaerolineae;Anaerolineales	67	18	37	46	22	10	103	9	7	42	21	11	12	22	32	74
Chloroflexi;Chloroflexia;Chloroflexales	18	40	23	28	14	40	51	7	1	44	7	28	20	20	15	48
Chloroflexi;Gitt-GS-136;bacterium	0	14	4	7	6	10	32	1	1	39	1	7	0	1	2	20
Chloroflexi;JG30-KF-CM66;bacterium	8	2	13	10	12	1	4	1	0	2	0	1	3	3	11	1
Chloroflexi;KD4-96;uncultured bacterium	11	20	10	14	16	33	55	2	10	64	1	16	28	9	12	18
Chloroflexi;Ktedono;C0119	7	26	8	19	20	77	22	39	25	39	25	29	51	52	22	22
Chloroflexi;S085;uncultured bacterium	30	3	4	1	4	4	10	0	4	19	0	1	13	2	2	9
Chloroflexi;TK10;uncultured bacterium	9	8	4	5	13	14	10	14	7	14	2	10	3	2	3	8
Chloroflexi;Thermomicrobia;AKYG1722	1	3	7	2	0	5	21	0	1	16	0	1	1	1	0	6
Chloroflexi;Thermomicrobia;JG30-KF-CM45	5	7	8	13	6	27	34	1	14	29	7	9	7	10	9	26
Chloroflexi;Thermomicrobia;Sphaerobacterales	1	1	0	0	5	7	8	4	2	5	7	8	16	0	1	7
Chloroflexi;uncultured;uncultured bacterium	12	5	5	7	2	5	24	1	4	20	4	1	10	0	4	3
Chloroflexi;uncultured;sludge bacterium A31	3	1	2	2	0	0	1	0	0	0	0	0	1	0	0	2
Cyano;Cyano;SubsectionIV	18	38	5	0	1	11	2	4	3	7	1	7	1	0	1	5
Firmicutes;Bacilli;Bacillales	136	156	132	127	37	302	44	93	1000	391	650	577	275	175	262	379
Firmicutes;Clostridia;Clostridiales	19	18	22	26	0	18	9	7	65	64	54	65	30	36	12	45
Firmicutes;Clostridia;Thermoanaerobacterales	2	0	2	0	0	3	0	0	4	0	1	3	0	0	0	2
Firmicutes;Erysipelotrichia;Erysipelotrichales	0	12	23	0	0	3	7	2	9	10	10	50	5	14	1	15

Gemmatimonadetes;Gemmatimonadetes; Gemmatimonadales	57	62	69	111	173	48	18	91	4	27	42	12	106	81	98	14
Nitrospirae;Nitrospira;Nitrospirales	6	12	23	25	20	5	3	7	2	12	3	2	1	7	4	0
Proteobacteria;Alphaproteobacteria;Caulobacterales	2	10	5	3	1	11	13	10	8	1	4	1	5	3	0	3
Proteobacteria;Alphaproteobacteria;Rhizobiales	186	163	151	130	127	64	80	152	14	26	51	32	98	117	149	86
Proteobacteria;Alphaproteobacteria;Rhodospirillales	27	42	48	29	59	22	30	40	4	16	20	13	23	33	39	25
Proteobacteria;Alphaproteobacteria; Sphingomonadales	13	16	17	7	19	15	71	35	8	15	32	8	7	17	1	43
Proteobacteria;Betaproteobacteria;Burkholderiales	39	32	46	36	30	18	4	60	0	13	4	17	15	20	17	8
Proteobacteria;Betaproteobacteria;Nitrosomonadales	4	19	12	13	22	11	2	16	5	4	6	5	6	8	6	1
Proteobacteria;Betaproteobacteria;Rhodocyclales	2	1	1	4	11	0	0	8	0	0	0	0	0	0	0	1
Proteobacteria;Betaproteobacteria;SC-I-84	26	9	7	16	21	4	0	6	0	3	4	1	13	12	13	1
Proteobacteria;Betaproteobacteria;TRA3-20	6	2	5	8	3	1	9	7	0	0	0	0	0	0	3	1
Proteobacteria;Deltaproteobacteria; Desulfuromonadales	6	0	6	7	6	1	0	12	0	1	1	1	12	5	3	0
Proteobacteria;Deltaproteobacteria;GR-WP33-30	20	13	25	12	4	2	12	5	0	1	4	1	2	11	23	3
Proteobacteria;Deltaproteobacteria; Myxococcales	16	25	52	32	40	20	18	33	14	8	12	13	34	47	27	14
Proteobacteria;Gammaproteobacteria; Legionellales	8	13	6	20	11	3	1	51	0	1	2	0	22	10	21	5
Proteobacteria;Gammaproteobacteria; Methylococcales	0	0	0	1	0	0	0	0	0	1	2	0	3	0	0	0
Proteobacteria;Gammaproteobacteria;NKB5	2	1	0	1	2	1	0	11	0	0	0	0	1	6	6	0
Proteobacteria;Gammaproteobacteria; Xanthomonadales	58	59	60	79	31	84	12	161	14	8	26	17	51	51	26	17

Table S4a: Absolute counts OTUs assigned to bacteria classes (genomic DNA samples)

Taxon	cSLM_1	cSLM_2	cSLM_3	cSLM_4	cM_1	cM_2	cM_3	cM_4	cMA_1	cMA_2	cMA_3	cMA_4	cSL_1	cSL_2	cSL_3
Acidobact ria;Acidobacteria;Acidobacteriales	11	11	4	25	38	55	25	59	5	30	6	5	17	0	0
Acidobacteria;Acidobacteria;Subgroup 17	1	7	2	1	0	0	0	0	2	3	7	6	6	6	2
Acidobacteria;Acidobacteria;Subgroup 2	2	1	1	3	12	9	13	23	2	7	1	0	1	0	1
Acidobacteria;Acidobacteria;Subgroup 3	34	47	36	48	57	45	78	107	23	37	37	26	57	55	102
Acidobacteria;Acidobacteria;Subgroup 4	12	3	7	0	2	2	2	0	6	14	8	1	2	4	8
Acidobacteria;Acidobacteria;Subgroup 6	61	52	47	31	22	11	6	10	40	84	52	42	64	34	41
Acidobacteria;Holophagae;Subgroup 10	6	1	8	2	0	0	0	2	4	7	2	2	10	7	1
Acidobacteria;Holophagae;Subgroup 7	3	2	2	9	0	1	0	0	0	2	1	0	3	0	2
Actinobacteria;Acidimicrobiia;Acidimicrobiales	42	15	39	30	17	10	13	35	38	38	33	29	28	30	32
Actinobacteria;Actinobacteria;Coryneles	14	2	2	1	0	3	2	14	0	0	0	2	3	0	0
Actinobacteria;Actinobacteria;Frankiales	17	28	22	49	52	74	53	63	16	45	11	24	23	12	28
Actinobacteria;Actinobacteria;Micrococcales	272	228	217	317	207	50	42	54	290	95	165	278	142	162	102
Actinobacteria;Actinobacteria;Micromonosporales	22	22	21	16	14	18	10	9	7	4	4	6	8	12	7
Actinobacteria;Actinobacteria;Pseudonocardiales	3	4	12	5	0	10	0	4	3	12	1	2	2	0	0
Actinobacteria;Actinobacteria;Streptomycetales	13	12	9	10	12	14	51	36	2	24	14	0	8	6	25
Actinobacteria;Actinobacteria;Streptosporangiales	5	0	1	12	3	4	5	1	1	0	7	3	0	3	4
Actinobacteria;MB-A2-108;uncultured bacterium	0	0	5	4	3	2	0	0	0	1	0	2	1	0	0
Actinobacteria;Thermoleophilia;Gaiellales	48	41	30	64	103	94	126	106	20	92	47	24	37	17	68
Actinobacteria;Thermoleophilia;Solirubrobacterales	30	39	48	46	63	37	58	21	29	57	30	32	22	30	53
Bacteroidetes;Cytophagia;Cytophagales	1	2	2	3	0	0	0	0	3	0	2	1	2	1	1
Bacteroidetes;Sphingobacteriia;Sphingoles	16	15	11	14	19	11	7	3	16	8	32	10	14	9	13
Chlorobi;Ignavi;Ignaviles	1	0	0	1	2	2	0	6	7	0	2	0	4	1	1
Chloroflexi;Anaerolineae;Anaerolineales	27	24	30	22	16	9	2	2	29	22	33	23	31	18	28
Chloroflexi;Chloroflexia;Chloroflexales	22	12	15	10	18	0	6	3	8	27	18	5	14	13	52
Chloroflexi;Gitt-GS-136;uncultured bacterium	8	2	4	10	3	6	2	1	3	8	1	7	13	7	8
Chloroflexi;JG30-KF-CM66;uncultured bacterium	5	5	6	2	7	3	7	6	4	1	3	0	4	2	13
Chloroflexi;KD4-96;uncultured bacterium	13	21	24	12	10	9	13	15	6	21	24	17	28	20	22
Chloroflexi;Ktedono;C0119	8	4	7	14	17	9	9	2	1	34	5	1	16	1	15
Chloroflexi;S085;uncultured bacterium	0	2	5	4	4	1	2	2	1	11	8	7	11	2	8
Chloroflexi;TK10;uncultured bacterium	9	10	4	5	9	10	8	4	0	10	9	4	4	8	11
Chloroflexi;Thermomicrobia;AKYG1722	3	1	2	1	0	1	0	0	0	1	2	0	0	1	9
Chloroflexi;Thermomicrobia;JG30-KF-CM45	5	2	6	8	5	8	1	0	1	6	0	10	5	6	22
Chloroflexi;Thermomicrobia;Sphaerobacterales	0	0	0	3	1	4	7	6	0	0	0	0	0	0	0
Chloroflexi;uncultured;uncultured bacterium	1	1	1	0	1	2	0	0	0	2	0	1	0	0	1
Cyano;Cyano;SubsectionIV	0	2	5	27	3	3	1	1	26	5	12	16	13	28	11
Firmicutes;Bacilli;Bacillales	289	293	93	163	209	221	328	237	88	93	153	85	84	165	163
Firmicutes;Clostridia;Clostridiales	7	10	5	24	13	5	7	4	2	2	2	7	9	17	9
Firmicutes;Clostridia;Thermoanaerobacterales	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Firmicutes;Erysipelotrichia;Erysipelotrichales	0	1	1	7	0	3	0	1	1	0	0	2	1	2	3
Gemmatimonadetes;Gemmatimonadetes; Gemmatimonadales	35	27	17	23	25	37	36	42	12	51	25	9	20	21	28
Nitrospirae;Nitrospira;Nitrospirales	11	12	8	4	2	0	4	8	2	4	4	0	5	2	3

Proteobacteria;AlphaProteobacteria;Caulobacterales	7	4	13	6	15	30	20	22	12	4	10	16	12	26	12
Proteobacteria;AlphaProteobacteria;Rhizobiales	84	98	194	88	78	103	111	96	115	114	159	156	113	130	90
Proteobacteria;AlphaProteobacteria;Rhodospirillales	32	35	54	48	51	75	86	105	62	73	28	60	51	42	41
Proteobacteria;AlphaProteobacteria;Sphingomonadales	3	11	39	4	13	41	38	13	14	11	17	25	35	28	44
Proteobacteria;BetaProteobacteria;Burkholderiales	42	31	26	12	17	8	10	19	78	24	45	77	36	15	36
Proteobacteria;BetaProteobacteria;Nitrosomonadales	13	8	3	10	8	5	3	4	3	3	15	5	13	11	7
Proteobacteria;BetaProteobacteria;Rhodocyclales	0	1	0	0	0	0	0	0	3	0	0	3	0	0	0
Proteobacteria;BetaProteobacteria;SC-I-84	0	5	3	2	6	1	0	2	0	2	4	4	3	3	7
Proteobacteria;BetaProteobacteria;TRA3-20	1	1	0	4	0	1	1	0	2	4	3	1	7	3	9
Proteobacteria;DeltaProteobacteria; Desulfuromonadales	1	11	1	1	14	3	2	3	16	11	4	20	1	6	7
Proteobacteria;DeltaProteobacteria;GR-WP33-30	4	9	8	1	7	5	9	4	14	3	18	17	6	20	2
Proteobacteria;DeltaProteobacteria;Myxococcales	113	137	135	143	120	158	139	149	183	146	254	158	262	205	159
Proteobacteria;GammaProteobacteria;Xanthomonadales	27	28	133	41	57	77	73	83	79	51	24	90	85	79	35

Table S4b: Absolute counts of OTUs assigned to bacterial classes (cDNA samples)

Taxon	gSLM_1	gSLM_2	gSLM_3	gSLM_4	gM_1	gM_2	gM_3	gM_4	gMa_1	gMa_2	gMa_3	gMa_4	gSL_1	gSL_2	gSL_3	gSL_4
Acidobacteria;Acidobacteria; Acidobacteriales;Acidobacteriaceae	19	4	24	26	86	88	31	5	5	3	5	17	32	41	22	7
Acidobacteria;Acidobacteria; Subgroup 3;Unknown Family	50	34	34	60	58	96	20	15	14	2	3	6	53	42	26	6
Acidobacteria;Acidobacteria; Subgroup 6;uncultured Acidobacteria bacterium	41	16	40	13	4	19	2	10	4	6	2	3	25	8	11	1
Acidobacteria;Acidobacteria;Subgroup 6;uncultured Acidobacteriales bacterium	21	5	15	8	5	6	3	0	1	0	0	1	9	1	4	5
Acidobacteria;Acidobacteria;Subgroup 6;uncultured bacterium	37	24	52	49	25	83	13	8	12	13	2	5	76	15	42	17
Acidobacteria;Holophagae;Subgroup 10;ABS-19	11	8	10	6	4	8	1	18	1	7	0	0	3	2	3	5
Acidobacteria;Holophagae;Subgroup 7;uncultured bacterium	16	1	6	2	5	16	1	0	0	1	1	0	10	13	12	0
Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae	9	20	18	14	3	13	1	6	2	5	0	3	8	1	5	4
Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured	12	9	23	25	16	34	6	21	1	10	1	13	25	11	15	3
Actinobacteria;Actinobacteria;Micrococcales;Intrasporangiaceae	3	31	13	22	21	13	22	22	26	16	2	13	2	26	7	9
Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae	124	169	36	53	129	41	85	276	160	161	9	29	117	59	175	171
Actinobacteria;Actinobacteria;Micromonosporales; Micromonosporaceae	12	21	9	19	25	38	23	21	46	15	12	17	6	15	14	29
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae	5	35	21	26	18	26	24	24	34	26	12	21	11	35	34	23
Actinobacteria;Actinobacteria;Streptomycetales;Streptomycetaceae	8	9	13	8	6	18	9	5	10	6	5	9	10	13	21	5
Actinobacteria;Thermoleophilia;Gaiellales;Gaiellaceae	7	14	16	4	17	28	40	20	21	40	23	18	22	23	14	17
Actinobacteria;Thermoleophilia;Gaiellales;uncultured	30	16	18	33	50	71	113	64	85	40	78	121	44	65	47	40
Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae	39	2	19	10	2	0	2	0	0	6	1	0	7	5	4	5
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae	67	30	37	18	12	22	10	103	11	42	7	21	32	12	22	74
Chloroflexi;Chloroflexia;Chloroflexales;Roseiflexaceae	17	15	22	36	9	14	40	44	28	43	1	7	15	20	20	47
Chloroflexi;KD4-96;uncultured bacterium;Other	11	24	10	20	7	16	33	55	16	64	10	1	12	28	9	18
Chloroflexi;Ktedonobacteria;C0119;uncultured bacterium	7	6	6	16	16	20	71	22	27	35	24	23	19	44	45	15
Chloroflexi;S085;uncultured bacterium;Other	30	5	4	3	1	4	4	10	1	19	4	0	2	13	2	9
Cyanobacteria;Cyanobacteria;SubsectionIV;FamilyI	18	5	5	38	0	1	11	2	7	7	3	1	1	1	0	5
Firmicutes;Bacilli;Bacillales;Bacillaceae	101	77	93	78	59	35	204	26	398	244	768	478	202	195	137	274
Firmicutes;Bacilli;Bacillales;Planococcaceae	4	12	8	38	5	0	22	6	60	46	89	40	13	6	12	40
Firmicutes;Clostridia;Clostridiales;Clostridiaceae 1	1	0	2	3	6	0	10	8	6	4	41	16	3	4	5	13
Gemmatimonadetes;Gemmatimonadetes; Gemmatimonadales;Gemmatimonadaceae	57	17	69	62	75	173	48	18	12	27	4	42	98	106	81	14
Proteobacteria;Alphaproteobacteria;Rhizobiales;Bradyrhizobiaceae	7	49	12	15	25	0	14	14	2	1	1	5	10	5	10	2
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae	43	52	35	26	26	23	19	29	13	5	0	20	26	20	22	28
Proteobacteria;Alphaproteobacteria; Rhizobiales;Rhizobiales Incertae Sedis	23	9	17	18	43	12	4	3	1	0	0	12	38	14	8	7
Proteobacteria;Alphaproteobacteria; Rhizobiales;Rhodobiaceae	21	4	15	20	18	46	2	0	2	5	0	1	37	41	17	1
Proteobacteria;Alphaproteobacteria; hizobiales;Xanthobacteraceae	40	8	30	18	45	32	9	1	3	1	2	5	15	5	28	9
Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae	10	32	23	8	14	29	7	14	4	8	1	2	22	3	14	15
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadace	2	16	7	13	8	17	5	51	2	6	7	26	0	3	11	34
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae	17	17	29	9	13	8	10	0	3	4	0	1	11	13	11	3
Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae	20	3	17	21	15	22	2	3	7	7	0	2	4	2	9	3
Proteobacteria;Betaproteobacteria;Nitrosomonadales;	4	3	12	19	18	22	11	0	5	4	5	6	6	6	8	1

Nitrosomonadaceae																
Proteobacteria;Betaproteobacteria;SC-I-84;uncultured bacterium	10	2	3	7	23	13	2	0	0	1	0	4	4	13	10	1
Proteobacteria;Betaproteobacteria;SC-I-84; uncultured beta proteobacterium	12	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Proteobacteria;Deltaproteobacteria;GR-WP33-30; uncultured bacterium	19	7	20	11	15	3	2	12	1	1	0	4	23	1	9	3
Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacteraceae	7	20	11	0	3	14	1	5	1	0	0	0	10	15	6	2
Proteobacteria;Deltaproteobacteria;Myxococcales;Haliangiaceae	3	24	11	4	8	5	6	0	0	0	0	5	0	4	1	3
Proteobacteria;Deltaproteobacteria;Myxococcales;uncultured	1	46	14	5	5	1	3	3	1	2	11	2	3	2	4	4
Proteobacteria;Gammaproteobacteria;Xanthomonadales; Xanthomonadaceae	13	27	23	22	58	5	60	3	6	0	10	19	10	33	26	7
Proteobacteria;Gammaproteobacteria; Xanthomonadales;Xanthomonadales Incertae Sedis	5	13	6	14	1	0	16	1	9	0	1	4	1	1	11	1
Proteobacteria;Gammaproteobacteria;Xanthomonadales;uncultured	40	93	31	23	16	26	8	8	2	8	3	0	15	17	14	9

Table 5a: Absolute counts of OTUs assigned tobacterial r oders (genomic samples)

Taxon	cSLM_1	cSLM_2	cSLM_3	cSLM_4	cM_1	cM_2	cM_3	cM_4	cMa_1	cMa_2	cMa_3	cMa_4	cSL_1	cSL_2	cSL_3	cSL_4
Acidobacteria;Acidobacteria; Acidobacteriales;Acidobacteriaceae	48	34	32	45	56	44	72	105	23	34	34	26	98	53	49	23
Acidobacteria;Acidobacteria; Subgroup 3;Unknown Family	6	16	12	5	2	1	1	0	11	24	16	11	13	17	11	11
Acidobacteria;Acidobacteria; Subgroup 6;uncultured Acidobacteria bacterium	2	5	8	4	3	0	2	2	5	9	4	3	6	12	2	5
Acidobacteria;Acidobacteria;Subgroup 6;uncultured Acidobacteriales bacterium	23	24	37	42	16	8	3	8	23	51	27	24	21	33	20	23
Acidobacteria;Acidobacteria;Subgroup 6;uncultured bacterium	2	8	4	1	0	0	0	2	4	4	0	1	0	8	6	4
Acidobacteria;Holophagae;Subgroup 10;ABS-19	9	1	3	2	0	0	0	0	0	2	1	0	2	1	0	0
Acidobacteria;Holophagae;Subgroup 7;uncultured bacterium	9	20	6	3	6	5	2	0	25	7	6	10	6	11	12	25
Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae	17	9	33	11	11	4	11	35	10	28	24	12	22	8	11	10
Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured	61	31	49	39	97	19	23	23	44	63	36	26	48	50	17	44
Actinobacteria;Actinobacteria;Micrococcales;Intrasporangiaceae	254	169	218	185	98	26	13	17	233	29	129	245	51	87	123	233
Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae	16	21	22	22	14	18	10	9	7	4	4	6	7	8	12	7
Actinobacteria;Actinobacteria;Micromonosporales;Micromonosporaceae	42	35	40	38	35	26	24	16	52	86	36	37	76	58	61	52
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae	10	9	13	12	12	14	51	36	2	24	14	0	25	8	6	2
Actinobacteria;Actinobacteria;Streptomycetales;Streptomycetaceae	7	14	13	9	20	11	24	15	7	30	10	8	38	12	7	7
Actinobacteria;Thermoleophilia;Gaiellales;Gaiellaceae	57	16	35	32	83	83	102	91	13	62	37	16	30	25	10	13
Actinobacteria;Thermoleophilia;Gaiellales;uncultured	3	2	1	2	0	0	0	0	3	0	2	1	1	2	1	3
Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae	22	30	27	24	16	9	2	2	29	22	33	23	28	31	18	29
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae	10	15	22	12	18	0	6	3	7	26	17	5	47	14	12	7
Chloroflexi;Chloroflexia;Chloroflexales;Roseiflexaceae	12	24	13	21	10	9	13	15	6	21	24	17	22	28	20	6
Chloroflexi;KD4-96;uncultured bacterium;Other	14	6	8	4	16	9	9	2	1	31	5	0	14	13	1	1
Chloroflexi;Ktedonobacteria;C0119;uncultured bacterium	4	5	0	2	4	1	2	2	1	11	8	7	8	11	2	1
Chloroflexi;S085;uncultured bacterium;Other	27	5	0	2	3	3	1	1	26	5	12	16	11	13	28	26
Cyanobacteria;Cyanobacteria;SubsectionIV;FamilyI	141	77	265	268	186	174	287	191	55	80	137	52	148	74	114	55
Firmicutes;Bacilli;Bacillales;Bacillaceae	14	12	8	8	13	17	28	23	27	10	11	20	10	10	42	27
Firmicutes;Bacilli;Bacillales;Planococcaceae	8	0	5	7	11	3	4	2	1	2	1	0	1	2	9	1
Firmicutes;Clostridia;Clostridiales;Clostridiaceae 1	23	17	35	27	25	37	36	42	12	51	25	9	28	20	21	12
Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales; Gemmatimonadaceae	19	49	14	15	17	17	18	23	7	21	16	30	8	10	19	7
Proteobacteria;Alphaproteobacteria; Rhizobiales;Bradyrhizobiaceae	16	52	12	17	19	20	24	19	23	20	35	33	22	36	19	23
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae	5	9	15	6	7	15	8	12	30	8	9	12	4	13	16	30
Proteobacteria;Alphaproteobacteria; Rhizobiales;Rhizobiales Incertae Sedis	0	4	10	6	4	7	10	10	2	12	5	2	0	4	3	2
Proteobacteria;Alphaproteobacteria; Rhizobiales;Rhodobiaceae	16	8	1	5	18	8	28	16	6	15	16	17	5	14	8	6
Proteobacteria;Alphaproteobacteria; Rhizobiales;Xanthobacteraceae	10	32	10	14	8	17	11	15	20	26	13	30	20	13	18	20
Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae	3	16	3	4	12	37	32	11	5	10	7	11	27	23	18	5
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae	8	17	30	18	11	6	5	9	40	16	36	45	20	31	10	40
Proteobacteria;Betaproteobacteria; Burkholderiales;Comamonadaceae	1	3	11	9	5	1	4	5	34	4	7	31	2	0	4	34
Proteobacteria;Betaproteobacteria; Burkholderiales;Oxalobacteraceae	10	3	12	6	8	4	3	4	3	3	11	5	7	13	11	3
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae	2	2	0	2	5	0	0	1	0	0	4	3	6	0	3	0

Proteobacteria;Betaproteobacteria;SC-I-84;uncultured bacterium	0	0	0	3	0	1	0	1	0	0	0	0	0	0	0	0
Proteobacteria;Betaproteobacteria;SC-I-84; uncultured beta proteobacterium	1	7	1	9	2	3	9	2	7	2	15	12	1	6	14	7
Proteobacteria;Deltaproteobacteria;GR-WP33-30; uncultured bacterium	22	20	26	39	22	29	28	25	66	28	75	49	53	21	40	66
Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacteraceae	25	24	32	38	17	36	45	43	40	48	46	40	33	42	30	40
Proteobacteria;Deltaproteobacteria;Myxococcales;Haliangiaceae	34	46	21	22	32	35	13	20	37	39	51	27	29	141	61	37
Proteobacteria;Deltaproteobacteria;Myxococcales;uncultured	24	27	5	7	16	35	46	55	31	13	10	33	4	31	5	31
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae	3	13	3	5	11	5	3	1	17	8	3	15	12	8	22	17
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadales Incertae Sedis	14	93	19	16	29	33	24	22	29	29	11	34	19	46	52	29
Proteobacteria;Gammaproteobacteria;Xanthomonadales;uncultured	48	34	32	45	56	44	72	105	23	34	34	26	98	53	49	23

Table 5b: Absolute counts of OTUs assigned to bacterial orders (cDNA samples)

Taxon	gSLM_1	gSLM_2	gSLM_3	gSLM_4	gM_1	gM_2	gM_3	gM_4	gMa_1	gMa_2	gMa_3	gMa_4	gSL_1	gSL_2	gSL_3	gSL_4
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae (Subgroup 1);uncultured	19	16	21	25	56	87	18	5	5	3	3	5	23	23	17	4
Acidobacteria;Acidobacteria;Subgroup 3;Unknown Family;Bryobacter	17	11	13	36	28	26	5	4	11	1	1	5	22	10	9	2
Acidobacteria;Acidobacteria;Subgroup 3;Unknown Family;Candidatus Solibacter	33	26	21	24	30	70	15	11	3	1	2	1	31	32	17	4
Acidobacteria;Acidobacteria;Subgroup 6;uncultured Acidobacteria bacterium;Other	41	33	40	13	4	19	2	10	4	6	2	3	25	8	11	1
Acidobacteria;Acidobacteria;Subgroup 6;uncultured bacterium;Other	37	54	52	49	25	83	13	8	12	13	2	5	76	15	42	17
Acidobacteria;Holophagae;Subgroup 7;uncultured bacterium;Other	16	15	6	2	5	16	1	0	0	1	1	0	10	13	12	0
Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured bacterium	12	10	23	22	14	29	5	10	1	7	1	13	23	11	10	1
Actinobacteria;Actinobacteria;Micrococcales;Intrasporangiaceae;Knoellia	1	3	12	18	21	13	17	21	21	13	1	13	2	19	5	9
Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae;Arthrobacter	124	17	31	50	127	41	85	269	155	154	9	23	105	59	161	166
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae;Marmoricola	2	7	8	14	4	7	2	1	7	6	10	4	2	15	7	4
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae;Nocardioides	2	10	13	12	13	19	22	17	27	17	1	17	8	20	26	15
Actinobacteria;Actinobacteria;Pseudonocardiales;Pseudonocardiaceae;Pseudonocardia	3	14	0	6	15	21	2	6	14	5	6	1	4	5	15	8
Actinobacteria;Actinobacteria;Streptomycetales;Streptomycetaceae;Streptomyces	8	12	13	8	6	18	9	5	10	6	5	9	10	13	21	5
Actinobacteria;Thermoleophilia;Gaiellales;Gaiellaceae;Gaiella	7	9	16	4	17	28	40	20	21	40	23	18	22	23	14	17
Actinobacteria;Thermoleophilia;Gaiellales;uncultured;uncultured bacterium	26	22	17	32	44	65	101	61	82	39	75	119	41	61	45	37
Actinobacteria;Thermoleophilia;SolirubrobacteralesSolirubrobacteraceaeSolirubrobacter	0	5	4	3	5	6	4	9	5	7	3	0	0	3	5	0
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;uncultured	67	44	36	18	12	21	10	79	11	40	2	13	32	9	22	61
Chloroflexi;Chloroflexia;Chloroflexales;Roseiflexaceae;Roseiflexus	17	28	22	36	9	14	40	44	28	43	1	7	15	20	20	47
Chloroflexi;KD4-96;uncultured bacterium;Other;Other	11	14	10	20	7	16	33	55	16	64	10	1	12	28	9	18
Chloroflexi;Ktedono;C0119;uncultured bacterium;Other	7	15	6	16	16	20	71	22	27	35	24	23	19	44	45	15
Chloroflexi;S085;uncultured bacterium;Other;Other	30	1	4	3	1	4	4	10	1	19	4	0	2	13	2	9
Chloroflexi;TK10;uncultured bacterium;Other;Other	9	5	4	8	3	13	14	10	10	14	7	2	3	3	2	8
Firmicutes;Bacilli;Bacillales;Alicyclobacillaceae;Tumebacillus	25	14	7	22	21	2	26	1	44	47	35	40	19	61	15	23
Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	98	89	92	76	59	32	190	24	388	243	741	454	196	187	121	261
Firmicutes;Bacilli;Bacillales;Bacillaceae;Fictibacillus	1	5	0	0	0	1	11	2	0	1	11	6	5	8	15	5
Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Paenibacillus	6	3	16	3	9	0	29	7	37	20	51	40	13	5	6	23
Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;uncultured	1	3	1	29	3	0	4	5	32	35	39	17	5	5	4	23
Proteobacteria;Alphaproteobacteria;Rhizobiales;Bradyrhizobiaceae;Bradyrhizobium	0	1	1	5	2	0	15	0	11	7	42	17	6	0	5	9
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Devosia	3	1	0	1	0	0	1	0	10	1	2	3	1	0	1	1
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Filomicrobium	0	0	4	0	0	0	0	0	3	0	3	0	0	0	1	2
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Hyphomicrobium	0	0	2	2	0	0	0	0	4	3	2	3	0	1	1	5

Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae; Pedomicrobium	51	106	62	57	50	165	47	16	8	26	4	39	92	93	47	14
Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylobacteriaceae; Microvirga	4	11	9	14	25	0	14	14	1	1	1	5	10	4	10	2
Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae; Mesorhizobium	3	5	11	11	9	1	17	3	7	2	0	8	10	5	7	23
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiales Incertae Sedis;Bauldia	5	0	3	5	0	0	2	1	0	1	0	4	1	0	2	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae; Rhodobium	15	3	9	0	2	1	0	0	1	0	0	0	8	4	8	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae; uncultured	9	7	9	5	10	18	0	8	2	0	0	4	6	7	3	5
Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae; uncultured	11	8	11	18	3	1	4	14	2	6	7	0	11	3	4	9
Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillales Incertae Sedis;Reyranella	8	3	4	12	3	2	0	8	2	1	1	4	1	9	8	5
Proteobacteria;Alphaproteobacteria;Sphingomonadales; Sphingomonadaceae;Sphingomonas	17	10	7	8	5	1	0	3	0	0	0	4	25	12	3	3
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae; uncultured	21	20	15	20	18	46	2	0	2	5	0	1	37	41	17	1
Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae; Massilia	38	35	26	15	28	28	6	1	2	1	0	5	10	5	17	8
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;uncultured	6	5	14	6	6	26	0	5	0	2	0	1	21	1	5	7
Proteobacteria;Betaproteobacteria;SC-I-84;uncultured bacterium;Other	0	8	4	6	3	15	0	0	0	0	0	4	1	1	5	0
Proteobacteria;Betaproteobacteria;SC-I-84;uncultured betaproteobacterium;Other	1	0	1	1	3	2	3	46	0	4	5	26	0	0	9	33
Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Geobacteraceae;Geobacter	10	12	9	2	8	6	8	0	0	3	0	0	8	8	9	3
Proteobacteria;Deltaproteobacteria;GR-WP33-30;uncultured bacterium;Other	20	0	10	12	5	20	1	0	1	6	0	2	1	0	2	0
Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacteraceae; Anaeromyxobacter	4	11	12	16	16	22	10	0	5	3	4	6	6	6	7	1
Proteobacteria;Deltaproteobacteria;Myxococcales;Haliangiaceae; Haliangium	10	11	3	7	23	13	2	0	0	1	0	4	4	13	10	1
Proteobacteria;Deltaproteobacteria;Myxococcales;Polyangiaceae; Sorangium	12	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Proteobacteria;Deltaproteobacteria;Myxococcales;Sandaracinaceae;unculture d bacterium	3	7	5	0	5	6	1	0	1	1	0	1	2	10	4	0
Proteobacteria;Deltaproteobacteria;Myxococcales;uncultured;uncultured bacterium	19	4	20	11	15	3	2	12	1	1	0	4	23	1	9	3
Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;Aquicella	3	4	7	0	2	12	1	3	0	0	0	0	10	10	3	2
Proteobacteria;Gammaproteobacteria;Pseudomonadales; Pseudomonadaceae;Pseudomonas	3	7	11	4	8	5	6	0	0	0	0	5	0	4	1	3

Table S6a: Absolute counts of OTUs assigned to bacterial famiglie (genomic samples)

Taxon	cSLM_1	cSLM_2	cSLM_3	cSLM_4	cM_1	cM_2	cM_3	cM_4	cMA_1	cMA_2	cMA_3	cMA_4	cSL_1	cSL_2	cSL_3	cSLM_1
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae (Subgroup 1);uncultured	18	3	10	9	20	30	10	36	3	28	6	4	0	15	0	18
Acidobacteria;Acidobacteria;Subgroup 3;Unknown Family;Bryobacter	27	11	11	11	35	13	23	23	11	4	17	10	34	15	13	27
Acidobacteria;Acidobacteria;Subgroup 3;Unknown Family;Candidatus Solibacter	21	23	21	34	21	31	49	82	12	30	17	16	64	38	36	21
Acidobacteria;Acidobacteria;Subgroup 6;uncultured Acidobacteria bacterium;Other	6	16	12	5	2	1	1	0	11	24	16	11	13	17	11	6
Acidobacteria;Acidobacteria;Subgroup 6;uncultured bacterium;Other	23	24	37	42	16	8	3	8	23	51	27	24	21	33	20	23
Acidobacteria;Holophagae;Subgroup 7;uncultured bacterium;Other	9	1	3	2	0	0	0	0	0	2	1	0	2	1	0	9
Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured bacterium	17	6	32	10	11	4	10	33	4	21	17	11	20	4	7	17
Actinobacteria;Actinobacteria;Micrococcales;Intrasporangiaceae;Knoellia	46	29	49	37	91	17	21	18	37	53	34	22	44	36	16	46
Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae; Arthrobacter	244	166	206	176	90	22	11	14	232	28	119	242	50	83	117	244
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae; Marmoricola	17	21	15	15	23	13	12	7	33	62	24	22	52	35	32	17
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae; Nocardioides	23	11	25	21	11	13	8	9	19	17	11	15	22	20	25	23
Actinobacteria;Actinobacteria;Pseudonocardiales;Pseudonocardiaceae; Pseudonocardia	4	10	3	3	0	10	0	4	0	5	1	2	0	2	0	4
Actinobacteria;Actinobacteria;Streptomycetales;Streptomycetaceae; Streptomyces	10	9	13	12	12	14	51	36	2	24	14	0	25	8	6	10
Actinobacteria;Thermoleophilia;Gaiellales;Gaiellaceae;Gaiella	7	14	13	9	20	11	24	15	7	30	10	8	38	12	7	7
Actinobacteria;Thermoleophilia;Gaiellales;uncultured;uncultured bacterium	56	15	28	29	67	63	91	72	12	49	37	15	27	21	8	56
Actinobacteria;Thermoleophilia;Solirubrobacterals;Solirubrobacteraceae; Solirubrobacter	18	22	22	22	16	10	22	3	17	19	14	20	29	13	17	18
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;uncultured	21	30	25	23	12	1	2	2	29	21	30	23	28	29	18	21
Chloroflexi;Chloroflexia;Chloroflexales;Roseiflexaceae;Roseiflexus	10	15	22	12	18	0	6	3	7	26	17	5	47	14	12	10
Chloroflexi;KD4-96;uncultured bacterium;Other;Other	12	24	13	21	10	9	13	15	6	21	24	17	22	28	20	12
Chloroflexi;Ktedonobacteria;C0119;uncultured bacterium;Other	14	6	8	4	16	9	9	2	1	31	5	0	14	13	1	14
Chloroflexi;S085;uncultured bacterium;Other;Other	4	5	0	2	4	1	2	2	1	11	8	7	8	11	2	4
Chloroflexi;TK10;uncultured bacterium;Other;Other	5	4	9	10	9	10	8	4	0	10	9	4	11	4	8	5
Firmicutes;Bacilli;Bacillales;Alicyclobacillaceae;Tumebacillus	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	141	75	232	223	183	173	286	191	40	79	130	35	145	73	110	141
Firmicutes;Bacilli;Bacillales;Bacillaceae;Fictibacillus	0	2	30	44	0	1	0	0	15	1	7	17	3	1	2	0
Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Paenibacillus	3	2	12	14	7	23	5	12	2	3	3	8	4	0	8	3
Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales; Gemmatimonadaceae;uncultured	8	3	2	6	5	11	19	10	27	8	2	18	5	7	41	8
Proteobacteria;Alphaproteobacteria;Rhizobiales;Bradyrhizobiaceae; Bradyrhizobium	2	8	1	2	6	4	8	10	0	2	8	0	1	3	1	2
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae; Devosia	4	1	0	0	0	0	0	1	0	0	1	2	2	0	0	4
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae; Filomicrobium	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;	0	0	4	0	2	2	1	0	0	0	0	0	2	0	0	0

Hyphomicrobium																
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae; Pedomicrobium	18	8	33	12	20	30	31	33	12	46	21	8	22	15	20	18
Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylobacteriaceae; Microvirga	18	48	14	15	17	17	17	22	7	19	16	27	5	9	18	18
Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae; Mesorhizobium	8	17	4	2	9	7	17	8	12	1	4	14	3	14	5	8
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiales Incertae Sedis;Bauldia	0	3	0	1	0	0	0	0	2	1	10	1	1	3	6	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium	1	13	1	1	3	2	0	3	2	5	3	6	3	10	1	1
Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae; uncultured	4	13	3	8	2	0	3	2	1	6	15	7	10	7	1	4
Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae; uncultured	8	15	15	8	5	1	0	1	10	9	23	10	7	4	20	8
Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillales Incertae Sedis;Reyranella	4	17	5	10	6	4	11	5	5	13	18	8	22	5	19	4
Proteobacteria;Alphaproteobacteria;Sphingomonadales; Sphingomonadaceae;Sphingomonas	2	4	10	2	1	2	4	0	15	2	8	7	4	10	11	2
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae; uncultured	0	4	8	5	4	7	9	10	2	12	5	2	0	4	3	0
Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae; Massilia	10	8	1	3	13	3	22	14	6	13	15	15	4	12	7	10
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae; uncultured	1	7	6	6	5	8	3	5	11	14	3	6	0	1	7	1
Proteobacteria;Betaproteobacteria;SC-I-84;uncultured bacterium;Other	2	6	1	5	11	6	8	11	4	7	1	2	1	5	4	2
Proteobacteria;Betaproteobacteria;SC-I-84;uncultured betaproteobacterium;Other	3	11	3	4	12	37	32	11	5	10	4	10	27	21	18	3
Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Geobacteraceae;Geo bacter	3	7	17	10	4	3	4	6	19	10	11	22	7	22	3	3
Proteobacteria;Deltaproteobacteria;GR-WP33-30;uncultured bacterium;Other	1	1	6	6	4	0	1	1	22	1	1	20	2	0	0	1
Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacteraceae; Anaeromyxobacter	8	3	12	6	7	4	3	3	3	3	7	5	5	8	8	8
Proteobacteria;Deltaproteobacteria;Myxococcales;Haliangiaceae; Haliangium	2	2	0	2	5	0	0	1	0	0	4	3	6	0	3	2
Proteobacteria;Deltaproteobacteria;Myxococcales;Polyangiaceae; Sorangium	0	0	0	3	0	1	0	1	0	0	0	0	0	0	0	0
Proteobacteria;Deltaproteobacteria;Myxococcales;Sandaracinaceae; uncultured bacterium	1	1	1	8	14	3	2	3	7	11	4	17	7	1	6	1
Proteobacteria;Deltaproteobacteria;Myxococcales;uncultured;uncultured bacterium	1	7	1	9	2	3	9	2	7	2	15	12	1	6	14	1
Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;Aquicella	14	15	24	27	21	24	26	23	40	15	64	31	44	14	31	14
Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	25	24	32	38	17	36	45	43	40	48	46	40	33	42	30	25
Proteobacteria;Gammaproteobacteria;Xanthomonadales;uncultured; uncultured bacterium	15	25	8	13	31	26	23	33	12	4	14	10	13	21	23	15

Table S6b: Absolute counts of OTUs assigned to bacterial famiglie (cDNA samples)