# Influence of exogenous organic matter on prokaryotic and eukaryotic microbiota in an agricultural soil. A multidisciplinary approach

Daniela Pezzolla [a,1], Gianpiero Marconi [b,1], Benedetta Turchetti [b,d,1], Claudia Zadra [c], Alberto Agnelli [b], Fabio Veronesi [b], Andrea Onofri [b], Gian Maria Niccolò Benucci [b], Pietro Buzzini [b,d,*], Emidio Albertini [b], Giovanni Gigliotti [a]

[a] Department of Civil and Environmental Engineering, University of Perugia, Italy
[b] Department of Agricultural, Food and Environmental Science, University of Perugia, Italy
[c] Department of Pharmaceutical Sciences, University of Perugia, Italy
[d] Industrial Yeasts Collection DBVPG, University of Perugia, Italy

## ARTICLE INFO

## ABSTRACT

The effects on bacterial, yeast and fungal communities present within an agricultural soil treated with a pig slurry-derived digestate were studied using a multidisciplinary (biochemical and 454 pyrosequencing platform) approach.

Biochemical analyses showed a significant increase of $CO_2$ emissions from soil 5 days after the amendment with digestate, whereas soil microbial biomass (C-biomass) increased significantly only after 12 and 30 days. PLFAs analysis revealed a significant increase in Gram-negative bacteria 90 days after the amendment.

Results from 454 pyrosequencing revealed the presence of OTUs attributed to bacteria, yeasts and filamentous fungi. Proteobacteria, Bacteroidetes and Firmicutes exhibited a significant predominance in the first 5 days, whereas Ascomycota became predominant 90 days after the amendment.

Overall, both bacterial and yeast + fungal richness exhibited a decreasing trend from 0 to 90 days after the amendment. Canonical analysis of principal coordinates showed that the cumulative effect of amendment and incubation time explained approximately 45% and 36% of the total variance observed in the bacterial and yeast + fungal communities, respectively. The correlation among some bacterial and fungal OTUs suggested the probable existence of specific biological interactions among different phyla.

The results reported represent a picture of the changes of soil microbial diversity in relation with some agronomic practices, such as organic amendments.

© 2014 Elsevier Ltd. All rights reserved.

## 1. Introduction

Soil is a dynamic and heterogeneous habitat, harbouring metabolically active communities, composed of both prokaryotic and eukaryotic microorganisms. These communities are important markers of soil fertility and ecology, and catalyse almost all the biochemical reactions occurring in soil (Nannipieri et al., 2003; Zhang and Xu, 2008). Soil microorganisms are able to use both labile and recalcitrant organic compounds as growth substrates: therefore they play a fundamental role in the decay of soil organic matter (SOM) and nutrient cycling (Coleman and Crossley, 1996; Insam, 2001; Nannipieri and Badalucco, 2003; Fontaine and Barot, 2005; Blagodatskaya and Kuzyakov, 2008). In fact, changes in SOM decomposition after the addition of organic substances (priming effect) are strictly related to microbial metabolism (Jenkinson et al., 1985; Kuzyakov et al., 2000).

Soil microbial communities include specific taxa that quickly metabolise the easily available substrates after their addition into the soil (Moore-Kucera and Dick, 2008). This rapid metabolism promotes the turnover of bacterial biomass, especially of fast-growing microorganisms, namely r-strategists (Blagodatskaya et al., 2007). Other microorganisms, in particular fungi, increase SOM decomposition, thanks to the stimulation promoted by moribund bacteria, which release cytoplasmic components after

---

their cell lysis. These slow-growing microorganisms are predominantly k-strategists (Fontaine et al., 2003; Blagodatskaya et al., 2007; Kuzyakov, 2010). Based on these considerations, the impact of environmental conditions on the dynamics of soil microbial communities can alter the rates of decomposition of organic materials (Treseder et al., 2011).

It is well known that traditional culture-dependent analyses are insufficient to characterise the whole soil prokaryotic and eukaryotic microbiota (Hill et al., 2000). Therefore, a few auxiliary biochemical markers (Vance et al., 1987; Insam, 2001; Kramer and Gleixner, 2008) must be used, such as: determination of i) C-biomass; ii) soil enzyme activity; and iii) phospholipid fatty acids (PLFAs). In particular, the analysis of PLFAs have been used extensively to evaluate the composition of soil microbial communities and to detect changes in fungal and bacterial partitioning under different environmental conditions (Frostegård and Bååth, 1996; Zelles, 1999; Nannipieri et al., 2003; Kramer and Gleixner, 2008; Frostegård et al., 2011).

Moreover, the characterization of DNA extracted directly from soil by high-throughput molecular methods (next-generation sequencing, i.e. NGS techniques) is proving more and more valuable to provide better in-depth information on the whole composition of prokaryotic and eukaryotic microbiota (Zhang and Xu, 2008; Glenn, 2011). Among NGS techniques, the 454 pyrosequencing platform represents the first developed platform that was broadly and successfully used for high-resolution analyses of microbial communities of forest soils (Hartmann et al., 2012), human oral biofilms (Langfeldt et al., 2014), and even spacecraft hardware (La Duc et al., 2012).

With regard to agricultural soils, agronomic practices (e. g. organic amendments) may affect both microbial diversity and C content, which are strictly interdependent (Lal, 2002). The organic amendments of agricultural soils are currently achieved by the addition of exogenous organic matter in order to improve soil fertility, mainly in terms of structure, increase of SOM content, N and P availability and biological activity (Toyota and Kuninaga, 2006; Melero et al., 2007; Badalucco et al., 2010; Sousanna et al., 2010). The waste materials used as organic amendments (manure, animal slurries, residues from agro-food industry or water purification plants, urban organic wastes, etc.) are often treated before their use in order to produce energy (e.g. anaerobic digestion for biogas production) and/or to obtain more stabilised sources of organic matter (i.e. matter with an increased concentration of recalcitrant organic molecules while maintaining nutrient levels, with respect to the initial feedstock material) (Tambone et al., 2009). The residual product obtained after biogas production, namely digestate, can be recycled as soil fertiliser due to its high content of plant-available $NH_4$–N (Lehtomäki and Björnsson, 2006; Odlare et al., 2008; Möller, 2009; Gunnarsson et al., 2010; Johansen et al., 2013).

Despite the impact of organic amendments to soil microbial communities has been broadly investigated (Pérez-Piqueres et al., 2006; Calbrix et al., 2007; Johansen et al., 2013; Ng et al., 2014), little information is still available about the influence of livestock derived organic materials on both prokaryotic and eukaryotic microbiota (Pezzolla et al., 2013). Particularly, the following questions arise: i) What is the impact of soil amendment on the structure of both prokaryotic and eukaryotic communities? ii) Is it possible to hypothesise the existence of specific interactions between different bacterial and/or yeast + fungal taxa? Unfortunately, current literature does not help to elucidate these fundamental questions, because most of the studies published in the last fifteen years have treated this matter by using incomplete approaches, i.e. the exclusive use of biochemical markers (Peacock et al., 2001; Calbrix et al., 2007; Bastida et al., 2008; Odlare et al.,

2008; Tambone et al., 2009; Johansen et al., 2013; Ng et al., 2014), or the supplementary use of molecular tools, but restricted to bacterial DNA (Toyota and Kuninaga, 2006; Nocker et al., 2010; Odlare et al., 2011). As the result, little is known about the dynamics of whole microbiota, which gives an incomplete picture of prokaryotic and eukaryotic communities inhabiting the soil after amendment. Therefore, the use of multidisciplinary approaches, e.g. the combined use of biochemical markers and molecular tools (i.e. NGS), is becoming increasingly necessary.

Accordingly, the aim of this study was to investigate the effects caused by the addition of a digestate (derived from an anaerobic treatment of pig slurry) on bacterial, yeast and fungal communities present within an agricultural soil using both biochemical markers and 454 pyrosequencing.

## 2. Material and methods

### 2.1. Soil and organic material

The soil used in the experiment was collected from the Ap horizon (0—15 cm) of an agricultural site (Casalina, Perugia, Italy, 42°57′10″N, 12°23′29″E). The soil was classified as Typical Ustifluvent (Soil Survey Staff, 2010), with a silty-loam texture, alkaline pH (8.3), and 9.6 and 1.1 g kg$^{-1}$ of total organic C and total N content, respectively (Pezzolla et al., 2013).

The digestate was collected from a biogas producing plant (Milan, Italy). The liquid pig slurry was processed via a two-stage anaerobic digestion involving both acidogenic and methanogenic bacteria. After slurry digestion, the biogas contained between 40 and 70% methane (by volume) (Ince, 1998; Abbasi et al., 2012). The chemical characteristics of the digestate are reported in Table 1.

### 2.2. Soil incubation

Soil aliquots (200 g) were put into sterile glass jars (250 ml) and amended with digestate to reach a final N concentration equivalent to 340 kg ha$^{-1}$ (corresponding to an application dose of digestate equal to 1.4 g kg$^{-1}$soil on a dry weight basis), in agreement with the limits reported by the European Nitrates Directive for the protection of groundwater against pollution caused by nitrates from agricultural sources (91/676/EEC). Aliquots of undisturbed soil were used in parallel as controls (CT). Three replicates of each condition (DG soil samples, i.e. treated with digestate, and CT soil samples) were incubated aerobically at 25 °C from 0 to 90 days and continuously maintained by sterile water at 60% of water holding capacity to ensure good biomass activation. In order to follow the time course of physical, chemical and microbial parameters, both DG and CT soil samples were periodically checked.

### 2.3. Determination of $CO_2$ emission

The glass jars containing DG and CT soil samples were sealed by screw-caps equipped with inlet and outlet valves. A $CO_2$-free air flow (20 ml min$^{-1}$) was flushed into the jar inlet ports and the gas

**Table 1**
Main characteristics of the digestate used in the experiment. All determinations were carried out in triplicate.

| Parameter | Digestate |
| --- | --- |
| Moisture (%) | 97.0 |
| Total organic C (g kg$^{-1}$ dry weight) | 233 ± 4.0 |
| Total N (g kg$^{-1}$ dry weight) | 100 ± 5.2 |
| C/N ratio | 2.3 |
| Water-extractable organic matter (WEOC) (g kg$^{-1}$ dry weight) | 28 ± 1.1 |