



Comparing the effect of digestate and chemical fertiliser on soil bacteria



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ABSTRACT

Different fertilisation regimes, i.e. the use of inorganic or organic fertilisers used in agriculture, are thought to cause differential effects on soil bacteria. In this study, glasshouse experiments were used to test the effects caused by addition of inorganic fertiliser or digestate from sewage sludge on soil bacterial community structure and diversity assessed by pyrosequencing of the V1–V3 region of the 16S rRNA gene. Spring wheat (*Triticum aestivum* L., cultivar Paragon) was used as model crop and its growth (measured by total dry weight) was monitored as well as changes in soil nitrogen and phosphorous at three time points over 128 days. Overall, 40 different bacterial phyla were detected with Proteobacteria, Acidobacteria and Actinobacteria dominating the communities. Additionally, members of the Bacteroidetes, Gemmatimonadetes, Chloroflexi and WS3 were found in all samples. Members of the Planctomycetes, Firmicutes, Nitrospirae, candidate division SPAM and Armatimonadetes were found in all samples but at lower abundances. Within the phylum Proteobacteria the classes Alpha- and Betaproteobacteria were most prevalent. Over the course of the experiment, the major differences between treatments were observed for the Actino-, Proteo- and the Acidobacteria. Sequences related to the Planctomycetes, implicated in nitrogen cycling, decreased in all treatments during the course of the experiment. Statistical analyses revealed that mainly nutrient addition and plant growth influenced the bacterial community structure. The effects of the treatment itself could be attributed to different gain in wheat growth especially when the communities were compared at the end of the trial. Our results indicate that the usage of different fertilisers will not only affect the bacterial community by direct addition of nutrients, but also indirectly.

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1. Introduction

A growing human population, decreasing availability of arable land as well as change in land use and more unpredictable weather conditions due to climate change are increasing pressures on agricultural productivity (Banwart, 2011). Productivity in turn is strongly linked with soil microbial communities since they form a fundamental part in retaining healthy soils by nature of the strong relationship between soil microorganisms, nutrient cycling, plant growth and disease suppression (Garbeva et al., 2004; Van der Heijden et al., 2007; Maron et al., 2011). However, current farming practise affects microbial diversity in soil mainly leading to its reduction (Roesch et al., 2007). Specifically, a decrease in bacterial abundance was observed for crop rotation while continuous cultivation of certain crops resulted in changes in community

structure (Ng et al., 2012). It is thought that current use of inorganic fertilisers is a major deleterious influence on the intrinsic soil microbial community and has been shown to cause decline in soil organic matter (Kibblewhite et al., 2008) across all land use types in the UK (Bellamy et al., 2005). Specifically the addition of nitrogen can affect carbon transformations (Kibblewhite et al., 2008) thus adversely affecting the functioning of the microbial community with potentially considerable effects on specific functions. An example is methane metabolism: the diversity of methanotrophs is known to be lower in agricultural soils (Levine et al., 2011).

Recently, the use of digestate as a soil additive was assumed to be beneficial (Albuquerque et al., 2012), since it contains essential nutrients such as phosphorous. Phosphorous in particular is a finite and diminishing resource (Malik et al., 2012) and production entails significant environmental cost in transport and mining of raw materials. Digestate is produced via anaerobic degradation of wastes, mainly derived from food, crops (either purpose-grown or crop residues) or sludge from waste water treatment processes, the

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solid fraction of which can be rich in nutrients including nitrogen and phosphorous as well as organics such as cellulose.

Although standards to minimise hazards in digestate have been developed in various countries to control heavy metals and pathogens (Paavola and Rintala, 2008; BSI, 2010) and organic pollutants (Al Seadi, 2009), other potential effects of the use of digestate on soil have been neglected. These include the amendment of soil organic matter composition, overload with nutrients, for example nitrogen compounds, and changes in microbial community composition (Alburquerque et al., 2012; Tambone et al., 2009; Fuchs et al., 2008). The latter could be observed even at fairly low rates of biosolid addition from waste water treatment to semi-arid grassland (Sullivan et al., 2006). In particular, Gram-positive bacteria became more abundant when sewage sludge was added to a long term soil organic matter experiment (Börjesson et al., 2012), combined with substantial changes in the overall microbial community structure linked to heavy metal input from the sludge. Also tannery sludge resulted in strong community and physiological shifts (Nakatani et al., 2011). Therefore, considerations on the origin of sludge are of high importance. This is supported by differential responses of microorganisms to the use of organic manure as only fertiliser, which resulted in differential responses for bacteria and fungi favouring Gram-negative bacterial and fungal communities. In contrast, negative effects were observed for Gram-positive bacteria like actinomycetes (Zhang et al., 2012).

Apart from microbial diversity, experiments on the effects of digestate on the condition of soil used for crop production need to incorporate a measure of crop productivity. Such studies have found that, while similar yields of crop could be obtained using abiotic fertiliser or digestate (Haraldsen et al., 2011), a combination of manure and biofertiliser addition led to an increase in microbial biomass, carbon and nitrogen mineralisation, soil respiration and enzyme activities (Dinesh et al., 2010) as well as bacterial diversity (Gu et al., 2009). However, the addition of abiotic nutrients alone resulted in stressed soil microbial communities (Dinesh et al.,

2010). In our study, we tested differences in plant growth and soil bacterial diversity in relation to the addition of digestate or abiotic fertiliser. Nutrient regimes were based on recently reported use of abiotic fertiliser by UK farmers (Defra, 2010) and advice on the use of digestate from the British Environment Agency (2010) applying similar levels of nitrogen (Abubaker et al., 2013) as most significant contributor to plant biomass.

We hypothesised that different nutrient sources (i.e. organic versus inorganic fertilizer) would be a significant influence on the soil microbial community as well as plant growth. Plant dry weight, as a measure of growth, was measured alongside changes in the soil bacterial communities. Up to now, only a few studies have used molecular tools to compare changes in soil microbial communities following either conventional agricultural techniques or the use of digestate as fertiliser. We have applied pyrosequencing since this technology has the potential to unravel underexplored microbial diversity (Maron et al., 2011) which might play a crucial role for adaptations of a community under changing conditions. More specifically, we examined changes in soil bacterial community structure influenced by different nutrient additions namely the addition of digestate produced from sewage sludge and inorganic fertilisers using spring wheat (*Triticum aestivum* L., cultivar Paragon) as model crop. At the start of the experiment, the major nutrients N and P as well as the bacterial community composition were assessed which was repeated after 65 and 128 days including plant biomass as factor.

2. Materials and methods

2.1. Experimental design

A glasshouse experiment with a completely randomized block design including three treatments with two replicates each was established. Pots of 0.025 m³ were filled with an agricultural soil described as Sandstone Upland with known pesticide history. The latter was taken from Leamington Spa, UK (Lat=52.32939,

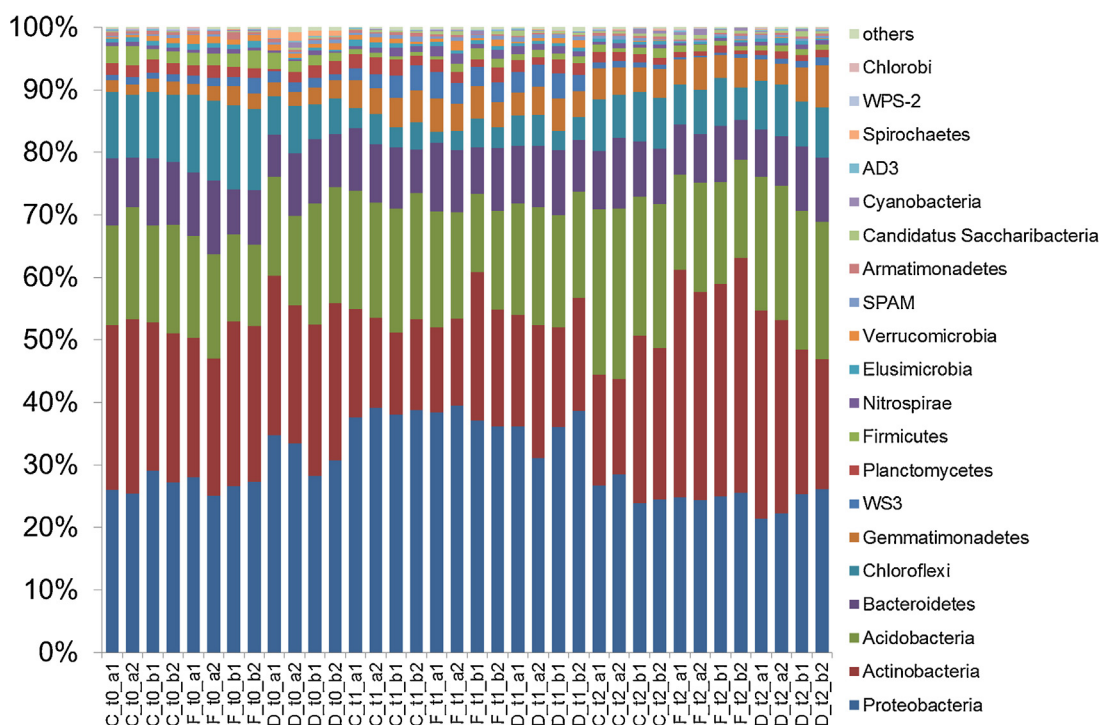


Fig. 1. Relative abundance of bacterial phyla assessed using 454 pyrosequencing. Phyla accounting for less than 0.03% were grouped into "others".

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