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Effects of fertilization with urban and agricultural organic wastes in a field trial – Prokaryotic diversity investigated by pyrosequencing

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ABSTRACT

The impact of different fertilizer treatments on prokaryotic diversity in a Danish urban waste field trial was investigated using tag-encoded amplicon pyrosequencing. The field trial was established in 2003 to investigate the application of urban organic waste as fertilizer in agriculture and to identify the effects on soil quality. The fertilizers (e.g. composted organic household waste, sewage sludge and human urine) contain a large amount of nutrients but possibly also undesirable toxic compounds that may influence the bacterial flora in the soil. A 561 bp fragment of the 16S rRNA gene flanking the V4, V5 and V6 regions, was amplified from each soil sample, tagged and sequenced using pyrosequencing. The major classified bacterial phyla and proteobacterial classes for all treatments were Actinobacteria, Acidobacteria and Betaproteobacteria, while the Crenarchaeota was the most frequent phylum of Archaea. No major changes in the community composition due to different fertilizer treatments were found, demonstrating a high robustness of the soil microbiota. However, some differences were observed e.g. Cyanobacteria were most frequent in the unfertilized soil, in comparison to the soils treated with nitrogen containing fertilizers and Firmicutes had higher occurrence in the soil with the composted household waste compared to all other treatments. Additionally, we used quantitative PCR (qPCR) to quantify specific bacterial groups, and used these numbers to convert the relative abundances of all bacteria obtained by pyrosequencing, to the actual numbers present in one gram of soil. All treatments resulted in a total number of bacteria between 1.99×10^9 and 4.11×10^9 gram^{-1} soil.

1. Introduction

Waste management systems of today were developed without primary concern for recycling (Magid et al., 2006a). However, present day urban areas increasingly represent a huge sink of nutrients (Faerge et al., 2001) and it may be argued that some of these (notably phosphate) should not be discarded as currently happens when sewage sludge and municipal household waste is incinerated or deposited in waste tips (Gilbert, 2009). In the past decades a significant change in composition of urban organic waste products has occurred in many first world countries, due to cleaner technologies as well as outsourcing of heavy industries. Due to recent regulation, the quality of the urban waste products may be expected to improve further. Thus, it is pertinent to examine the effects of contemporary urban waste on soil quality and function.

Composition of the microbial community in soil and induced changes caused by amendments, provide useful information on soil health and quality. The development of massive parallel pyrosequencing allows rapid analysis of microbial communities using 16S rRNA sequences. However, when sequencing relatively short 250 bp reads covering one of the hypervariable regions of the 16S rRNA, it is challenging to ensure enough information to thoroughly elucidate the diversity of the microbial community. Several studies have investigated whether taxonomic information based on full-length sequences can be recaptured by short reads covering different variable regions of the 16S rRNA produced by pyrosequencing (Huse et al., 2008; Liu et al., 2008; Youssef et al., 2009). It was found that the short reads provided equivalent taxonomy and relative abundances of microbial communities as full-length 16S rRNA sequences.

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The cost-benefit of diversity pyrosequencing has been increased by using tag-encoded amplicon pyrosequencing, where sample-specific sequence tags are incorporated on secondary amplification primers, enabling amplicons from individual samples to be pooled prior to pyrosequencing (Dowd et al., 2008b). Due to the large number of sequences provided by pyrosequencing, a very good resolution of the microbial diversity can be obtained, and reveal even small changes in microbial populations, which is unprecedented when comparing with previous fingerprinting techniques.

Amplicon pyrosequencing provides data on the relative abundance of specific bacteria in a sample. We proposed to use qPCR of specific bacterial groups, in combination with pyrosequencing, to obtain the actual number of all bacteria present in a sample. We chose to use the genus *Pseudomonas* and the class Flavobacteria as target for the quantification by qPCR as they are well known soil bacteria, making it possible to design appropriate primers. By relating the number of these two bacterial groups found by qPCR with the abundance of the same groups found through pyrosequencing, the total number of bacteria in one gram of soil was found. Having the total bacterial number, the abundances from pyrosequencing can be used to give the number of all bacteria belonging to any group present in the sample.

Interdisciplinary studies on integrated ecological waste management systems have encouraged the development of a long-term field trial under the CRUCIAL project (Magid et al., 2006b). The field trial was established to investigate options of recycling urban organic waste as fertilizer in agriculture and to identify the effects on soil quality. The soils have been treated annually with different urban and control fertilizers at normal and increased nitrogen levels. Among the fertilizers are human urine, sewage sludge and degassed and composted source-separated organic household waste. The key rationale for establishment of the field trial was, that by approaching the known limits in a 'realistic' way for a number of heavy metals (below which no profound disturbance should be observed on key soil ecological functions), it should be possible to discern if the many unknown components in the composite urban waste based fertilizers have measurable impacts on these functions. The working hypothesis is that the use of urban waste as fertilizers is not detrimental to soil function and microbial diversity in the long-term and even beneficial in the short to medium-term, as long as ecotoxicological limits for heavy metals are not exceeded.

It is well known that amendment with organic matter contributes to agricultural soil health by increasing nutrient levels, aggregation, biological activity and by reducing bulk density (Haynes and Naidu, 1998). However, it is also recognized that sewage sludge and other waste products can contain heavy metals, which may have detrimental effects when used as fertilizers in agriculture. In addition to the potential detrimental effects of undesired contaminants in the applied organic waste, it is of relevance to consider possible introduction of pathogenic or otherwise harmful microorganisms. The potential for spreading pathogens is important to investigate in relation to human health and to evaluate soil as a potential reservoir of resistance genes. Human urine does usually not contain pathogenic bacteria but studies show that source-separated urine may be contaminated with feces during separation and can contain potential pathogens (Schönning et al., 2002).

The objective of the present study was to investigate the impact of different fertilizer treatments on prokaryotic diversity in a Danish urban waste field trial using high throughput sequencing. The fertilizers contain high levels of nutrients but possibly also unwanted toxic compounds that may influence the microbial flora in the soil. In a separate paper (Poulsen et al., submitted for publication) we investigate the effect of the fertilizer treatments on soil parameters e.g. soil organic matter, basal CO_2 respiration and soil microbial biomass.

2. Materials and methods

2.1. Field site

The soil samples were collected from the field site situated at the experimental farm of the University of Copenhagen, 20 km west of Copenhagen, Denmark (55° 40′ N, 12° 18′ E) (Magid et al., 2006b). It includes 11 treatments on 33 plots of 891 m^2 each, in a random block structure. Each plot is separated from the neighboring plots by a 3 meter wide strip of grass, in order to avoid movement of soil between treatments. The site was established in 2002 and based on initial soil analysis it was decided to reduce the plot size resulting in a relatively uniform experimental field with respect to organic-C and texture (Magid et al., 2006b). Thus, the field trial has run in its present form since 2003.

The soils have been treated with different urban waste and reference fertilizers using application rates adjusted to supply a modest input of nitrogen (equivalent to approximately 100 kg N ha^{-1} $year^{-1}$ depending on the crop grown) using single sources and thus annual application rates were adjusted to take mineral fertilizer equivalents (MFE) into account. MFE is an empirical measure of the nutritional effect from organic manuring in comparison to mineral fertilizer that is used in Danish agriculture and based on Danish legislation (Anon, 2011) for regulation of maximum permissible fertilization use. Furthermore we introduced accelerated treatments aiming at three times the normal N level.

Soils from 6 different treatments were investigated: compost (accelerated level), sewage sludge (accelerated level), cattle manure (accelerated level), human urine (normal level), NPK inorganic fertilizer (normal level) and unfertilized. The fields were amended in springtime every year and grown with spring cereals. Except for the unfertilized control, all plots were undersown with clover. The fertilizers were spread on land and incorporated by ploughing to approximately 20 cm. The compost was produced by a private company receiving source separated organic waste from households. The compost had been degassed and subsequently composted and stabilized before use and hereafter called MSW-compost. The sewage sludge was from a public treatment plant receiving mixed wastewater from industries (not hazardous) and private households.

2.2. Soil sampling

Soil samples were collected in January after running the field experiment for 4 years. The time of sampling ensured that there were minimal effects of the most recent fertilization episode (April 2006), and that possible effects observed would be representative of the 4 year amendment history. From each of the 18 plots soil was collected from the top 20 cm by taking 15 sub samples with an auger and subsequent pooling the sub samples from each plot. The soil was spread on a table overnight to dry at room temperature and sieved through a 5 mm sieve and stored at −20 °C.

2.3. Determination of soil properties

Soil samples were dried at 60 °C overnight for determination of dry weight. Total content of C and N was determined by isotope-ratio mass spectrometry (IR-MS) (ANCA NT System, Europa Scientific). For all soil samples pH was measured by suspending 10 g of oven-dried soil in 25 ml 0.01 M $CaCl_2$ and stirred 5 times within an hour. The suspension was allowed to settle for 10 min before pH was measured.