EFFECT OF COMPOST APPLICATION ON THE SOIL MICROFLORA

JOEKE POSTMA AND HANS KOK

Plant Research International, Wageningen, The Netherlands

Introduction

Compost is used in agriculture as a fertiliser or to improve the physical structure of the soil. In addition, compost amended soil has been found to be suppressive against plant diseases caused by nematodes, bacteria, or soil-borne fungi in various cropping systems (Hoitink and Fahy, 1986; Ringer, 1998). However, the opposite, an increase of disease incidence due to a compost application, has also been demonstrated (Tuitert et al., 1998). Several reasons for these inconsistent results can be given. There are many types of compost, i.e. they differ in composition of organic materials and in maturity. In addition, the method and time of the compost application is important for the final effect in the field. Application of organic material just before planting the crop can even stimulate pathogens. Up to now, it is still difficult to predict the beneficial effect of compost products in plant pathosystems. To understand the influence of compost applications on soil suppressiveness, the microflora in soil before and after compost application has to be studied in more detail.

Compost is known to be a product rich in microbes, often containing 108 to 2 x 109 colony-forming units (CFU) of aerobic bacteria per gram dry matter (Postma et al., in press). However, the allowed application dosage of compost to agricultural soils is limited in the Netherlands (12 ton/ha per every second year, which is approx. 1 % w/w). Since agricultural soils themselves contain between 107 and 108 CFU aerobic bacteria per gram, the number of micro-organisms which are added through compost to a soil is not very impressive. However, the composition of the microflora in compost might be different, and the soil will be enriched with a variety of nutrients that will become available for the indigenous microflora.

Methods to characterise the microflora

To study the soil microflora, several methods are available which all give information about different aspects of that microflora. Thus, methods can yield quantitative versus qualitative data, can report on cultivable organisms versus total based on DNA (including dead organisms), or can describe specific functions present within the microflora. None of the available methods is able to give a complete explanation of the suppressive potential of the soil microflora. Therefore, a strategy of combining different types of methods is generally used.

A classical method is to compare the numbers of specific groups of cultural bacteria and fungi using plate counts on (semi)selective agar media. Many groups can be distinguished, such as fluorescent pseudomonads, filamentous actinomycetes, bacilli, etc. However, only the cultivable fractions of the soil population, which is often only 1-10 % of the total population, are thus analysed.

The microbial community structure and diversity can be analysed at the genetic level by PCR-DGGE community profiling (polymerase chain reaction - denaturing gradient gel electrophoresis).

This method allows the genetic fingerprinting of microbial populations in complex substrates with different levels of suppressiveness (Postma et al., 2000). PCR-DGGE profiles can be prepared with a focus on bacterial or fungal populations. Using nested PCR, also specific groups such as actinomycetes, pseudomonads, and bacilli can be analysed. With this technique, the non-cultivable fraction of the soil microflora is included in the profile. In addition, the possibility to identify bands in the profile following a cloning and sequencing procedure is very important, as this leads to a possible identification of the organisms present in the community.

The microbial population can also be analysed at a functional level with CLPP (community-level physiological profiling) using BIOLOG microtiter plates which contain 95 or 31 different single carbon sources. Microbial populations differ in their ability to use different carbon sources. By using BIOLOG microtiter plates the substrate utilisation pattern of the microbial community can be analysed (Garland and Mills, 1991; Waldrop et al., 2000). However, the results reflect the potential present in the microflora to utilise the different substrates, and not their in situ activity.

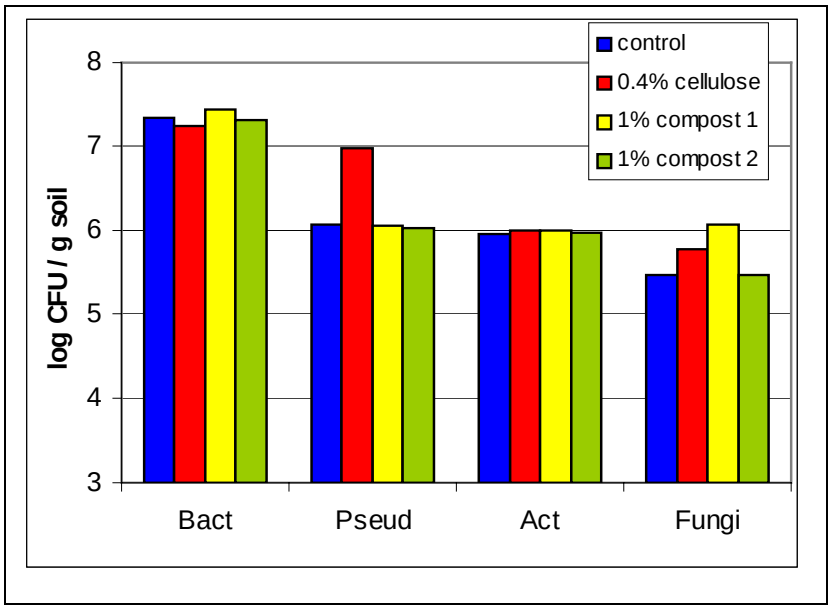
Furthermore, microbial activity can be analysed by different (fluorescent) staining techniques (TTC, FDA, CTC) or by measurement of soil respiration which gives information about the in situ activity.

A completely novel approach would be the use of DNA microarrays to report on the microbial status of complex substrates. Environmental DNA or RNA is hybridised to a microarray which contains a pre-selected range of DNA probes that are able to report on soil functional status. Problems to be solved with this highly innovative approach are a likely lack of sensitivity as well as cross-hybridisation.

Data on the soil microflora before and after compost application obtained with three of the above described methods will be discussed in the next paragraph.

Effect of different organic matter amendments on the soil microflora – a case study

Three types of organic matter, i.e. 0.4 % paper cellulose, 1 % spent mushroom compost and 1 % green-waste compost were mixed through a sandy soil, and incubated for 14 days at 18 °C. Then the microflora was characterised by 1) plate counts on semi-selective media, 2) ability to use the 31 carbon sources in Ecoplates (BIOLOG) and 3) genetic community profiling with PCR-DGGE. Results are shown in Figs. 1 to 3.

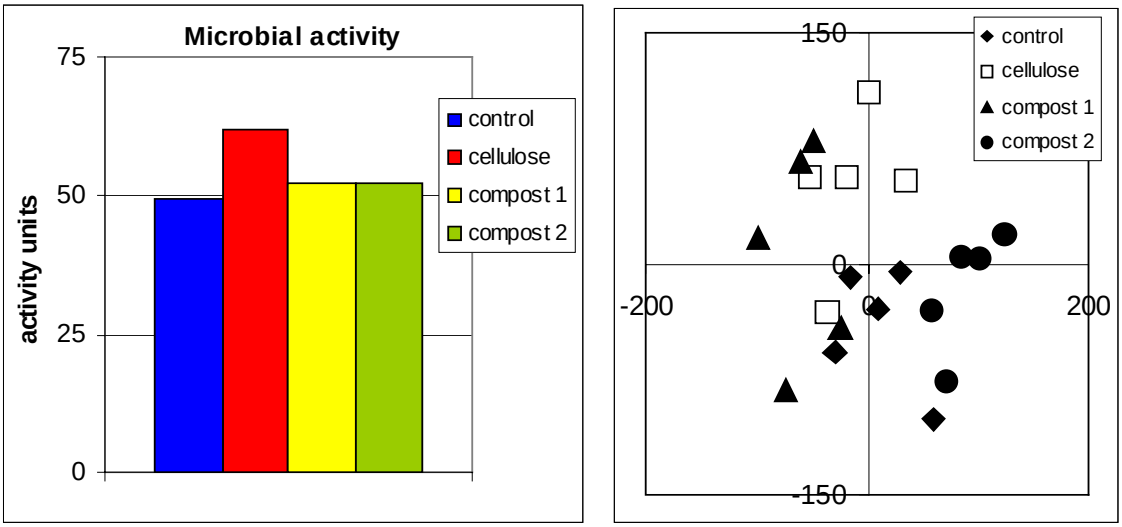


no amendment (control), 0.4 % paper cellulose (cellulose), 1 % spent mushroom compost (compost 1), or 1 % green-waste compost (compost 2). The enumerated micro-organisms are: total aerobic bacteria (Bact), fluorescent pseudomonads (Pseud), filamentous actinomycetes (Act), and fungi (Fungi). Least significant differences at P=0.05 are respectively: 0.16, 0.14, 0.19 and 0.12.

Fig. 1. Number of colony-forming units (CFU) of different groups of micro-organisms present in soil after amendment with different organic materials

Plate counts (Fig. 1) showed no differences for the total number of aerobic bacteria and filamentous actinomycetes, numbers of fluorescent pseudomonads increased only in the case that cellulose was amended, and numbers of fungi increased by amendment with cellulose as well as spent mushroom compost.

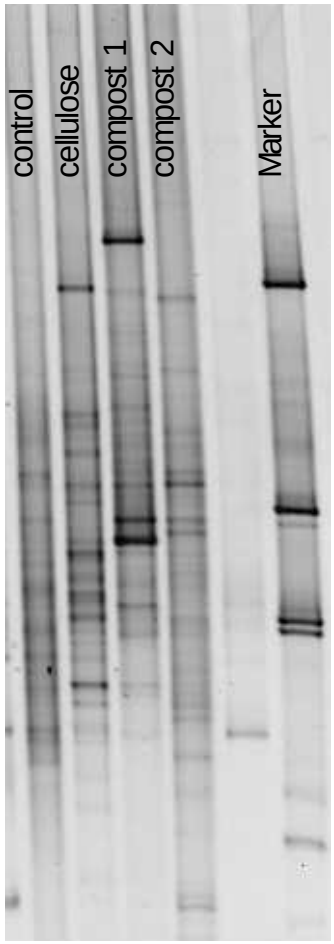
The potential microbial activity of the microflora in soil (Fig. 2A) was similar for most of the treatments, except for the cellulose amended treatment which had an increased potential activity.



no amendment (control), 0.4 % paper cellulose (cellulose), 1 % spent mushroom compost (compost 1), or 1 % green-waste compost (compost 2). A: Mean value of the reactions with 31 C-sources in the Eccoplates. B: Principal component analysis.

Fig. 2. Microbial activity measured in soil after amendment with different organic materials

However, principal component analysis of the BIOLOG data (Fig. 2B) showed a clear shift in the reaction of the microbial populations in soil after the different amendments. The cellulose and spent mushroom compost amended soil were different in their ability to use different carbon sources compared to the control and green-waste compost amended soil.



The genetic profiles of the bacterial populations in soil (Fig. 3) were distinctly different in all treatments. The control treatment had many week bands. In the treatments with the organic matter amendments few strong bands (depending on the type of organic matter) appeared. This means that some micro-organisms became more dominant, i.e. were stimulated by or introduced with the amendment, showing a decrease in species evenness.

Fig. 3. Genetic profile of the bacterial populations with PCR-DGGE in soil after amendment with different organic materials: no amendment (control), 0.4 % paper cellulose (cellulose), 1 % spent mushroom compost (compost 1), or 1 % green-waste compost (compost 2). The marker is composed of five known bacterial isolates.

Comparing the results of the different methods bring us to the following conclusions: the total number of cultivable micro-organisms did not increase by amendments, but the composition of the microflora was distinctly influenced in its genetic composition as well as in their ability to use different carbon sources (functional property). These microbial shifts occurred as a result of rather low concentrations of the amendments (i.e. 0.4 or 1 % w/w), and were dependent on the type of organic amendment.

In further studies, the type of organisms that are stimulated by the different types of organic amendment should be studied in relation to the effect on the crop.

Correlation between microflora and disease suppression

Different microbial characteristics have been analysed in relation with disease suppressiveness of different soil types or treatments (Alabouvette et al., 1985; Oyarzun, 1994; van Bruggen and Semenov, 2000; van Os and van Ginkel, 2001). Although microbial activity and biomass have been correlated with disease suppression (nutrient sink in suppressive soil), these community-level characteristics could not fully explain the level of disease suppressiveness. Van Os and van Ginkel (2001) showed that growth rate of a pathogenic Pythium species through differently treated soil, correlated negatively with dehydrogenase activity, microbial biomass as well as $^{14}CO_2$ respiration of the soil. However, a lower growth rate of the fungus through the soil was not always followed by a lower disease incidence level in the crop.

For disease suppression more properties of the microflora are suggested to be important: microbial diversity and the presence of specific suppressive micro-organisms or consortia might be necessary. Several studies showed a correlation between the presence of actinomycetes and disease suppression in soil or compost (Oyarzun, 1994 ; Workneh and van Bruggen, 1994; Craft and Nelson, 1996; Tuitert et al., 1998). Actinomycetes are key organisms in the decomposition of various organic substances and they are important producers of antibiotics, vitamins and many enzymes. Stimulation of actinomycetes after compost application as an explanation of increased soil suppressiveness is therefore an interesting hypothesis for further studies. Actinomycetes are only one example of a microbial group that might influence disease suppression. Other organisms responsible for disease suppression, in particular those of the non-cultivable fractions of soil micro-organisms, can be detected with molecular techniques such as PCR-DGGE.

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