Long Term Effects of Biosolid Applications on Soil Biological Parameters

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Abstract

For more than 15 years, there has been a progressive reinforcement of legislation related to biosolid utilization in the EU, particularly in France. There is a particular concern about effects on soil organisms, even though sludge spreading has been shown to have a favorable effect on soil biological characteristics by stimulating microbial activity and biomass [1], [2]. Generally, soil amendment of organic matter via sludge application also favors soil invertebrates and abundance of Carabidae, earthworms, nematodes, and mesofauna [3]. However, the responses of organisms to sludge application are specific. Changes in microbial and invertebrate community structure such as a decrease of species and functional diversity was also observed, especially if sludge is contaminated by heavy metals [2], [4].

The present results aimed to assess effects of different biosolids, including sewage sludge, composts, coal ashes and household waste ashes on soil biota. Each type of biosolid was applied manually to each plot (40 m^2) according to regulations (four applications of 10 t ha^{-1} within 10 yr, representing 160 kg of dry matter for each plot). Spreadings have stopped in 2002, and soil biological assessment was carried out in 2010. Biological indicators included bacterial diversity, enzymatic activity, microarthropods and macrofauna abundance and diversity. Some functional indicators, including biostructures, were also studied.

Pedologic and agronomic effects have shown the positive effect of biosolids on soil fertility, without serious contamination by heavy metals or organic pollutants [5]. The present results concerning soil biota show no significant effect on soil biota and functional parameters measured.

Introduction

There has been a gradual strengthening of related legislation in the EU, particularly in France, for the last 15 years concerning biosolid utilization in Agriculture. During that period, the quantity of sludge has steadily increased; from 800 kt in 2000 to 950 kt (dry wt.) today. In France, the three main methods of sludge disposal are incineration, land-filling and, most important, agricultural land application (60 %).

Numerous case studies have explored the potential positive and/or negative effects of biosolid application on soil quality. Most of the benefits concern soil fertility. Biosolids in general, and sludge in particular, are rich in nutrients, calcium, and organic matter and their application is known to have more or less long-lasting, favourable effects, on both biomass production and chemical and physical soil properties. Sludge spreading also has a favourable effect on soil biological characteristics by stimulating microbial activity and biomass [5].

The main risks concerning biosolids application are associated with soil pollution, particularly transfer of mineral and organic pollutants within the agro-system. It has been previously demonstrated that with high amounts of sludge application, possible transfers of pollutants, mainly trace elements, from soil to plants are possible [6]. Furthermore, concerns about the behaviour of organic pollutant, particularly PAHs and PCBs, coming from sewage sludge have been raised [7]. These conclusions have been recently summarized in several reviews showing that sewage sludge used in recommended

doses could usually increase fertility and yield, whereas the bioavailability of metals increases only in sludge amended soil at excessive rates of application over many years [8], [9], [10].
However, long-term experiments on the effects of biosolids on soil organisms are still needed, especially in contexts using the recommended biosolid doses for agriculture. Furthermore, to our knowledge, no long term study has been carried out to assess the effects on soil biota when spreading has stopped for a long period.
Our aim here was to assess the long term effects of eight biosolids on biological diversity and activity of soil organisms, after 10 years of biosolid application (1996 to 2002) followed by 8 years of no biosolid application.

Material and Methods

Field experiment

Experiments were carried out on the experimental farm at "La Bouzule" (12 km east of Nancy, France) [5], [11]. Ten treatments were applied to experimental plots. The following six different biosolid types were applied: liquid sewage sludge (LSS), lightly dehydrated sewage sludge (LDSS), lightly dehydrated composted sewage sludge (LDCSS), lightly dehydrated composted sludge with added organic pollutants (LDCSSO), lightly dehydrated composted sludge with added metals (LDCSSM), mixed paper sludge (MPS). Additionally, two other types of wastes were tested: coal ashes (CA), and household waste ashes (HWA). For convenience, these two other types of wastes will be also called "biosolids" throughout this paper. The two control plots did not receive any biosolids, but were subject to mineral fertilization. The first control (CMF) received a minimal fertilization sufficient only to avoid plant nutrient deficiency, while the second control (COF) received a higher and optimal quantitative fertilization, which corresponds to the doses usually applied in agriculture and allowing optimal yield [5]. A split block and plot design was created, including four plots (10 x 4 m) per treatment. Thus a total of 40 plots, including the 10 treatments, were carried out. Each type of biosolid was applied manually to each plot according to regulations (four applications of 10 t ha^{-1} within 10 years between 1996 and 2002, representing 160 kg of dry matter for each plot). For all the plots, biosolids were complemented with a mineral fertilization corresponding to the level of COF treatment. Between 2002 and 2010, all the plots were conducted like COF plots. Furthermore 4 supplementary "control" plots (TEMOIN) that have never been cultivated before 2008 were included in the sampling protocol.

Soil biota sampling

Bacterial communities, enzymatic activities, soil microarthropods, soil macrofauna, earthworms and samples for organo-mineral associations have been collected during springs 2010 and/or 2011 (depending of the parameter). Concerning Bacteria, total DNA was quantified using classical method of DNA extraction [12], purification and qPCR quantification [13]. Furthermore specific genes used as markers for atrazine degradation were quantified. On the other hand, the structure of bacterial communities was studied using the fingerprint method A-RISA. Enzymatic activities were studied under laboratory conditions by measuring soil potential degradation with Fluorescein Diacetate hydrolysis Assay (FDA). Microarthropods, including Acari and Collembola, were sampled on each plot using classical soil extraction methodologies previously described [14], [15]. Edaphic macrofauna was studied by collecting soil monoliths (25 x 25 x 15 cm) on each plot and manually extracting all Arthropods. These Arthropods were identified at the family level except for 3 groups identified at the species level: Oligochaeta, Isopoda and Chilopoda Lithobiomorpha. The biodiversity of Edaphic macrofauna was assessed by calculating the abundance of each taxonomic unit and the diversity (Shannon and Pielou indexes) on identified taxonomic units. Furthermore, earthworms were collected using formaldehyde extraction [16], [17]. Organo-mineral associations stability was assessed by granulo-densimetric fractionation methods, as well as micro-structures description using TEM (Transmission Electronic Microscopy) [18].

Results and discussion

The total abundance of microbial community varied between $1.25.10^9$ to $2.83.10^9$ copies of 16S rDNA. ANOVA statistics revealed that only TEMOIN plots were significantly different from other treatments, with a greater abundance. Furthermore no effect of the different treatments was detected upon functional communities degrading atrazine. However, even though the absence of atrazine application since 2004, the results show the presence of a resilient community able to degrade this herbicide. On the other hand, bacteria community structure analyzed by A-RISA did not show significant difference between treatments (figure 1). Enzymatic activities ranged between 17.9 to 22.3 µg of fluorescein /g. dry soil with no significant differences between treatments, except TEMOIN with values reaching 47.3 µg/g.

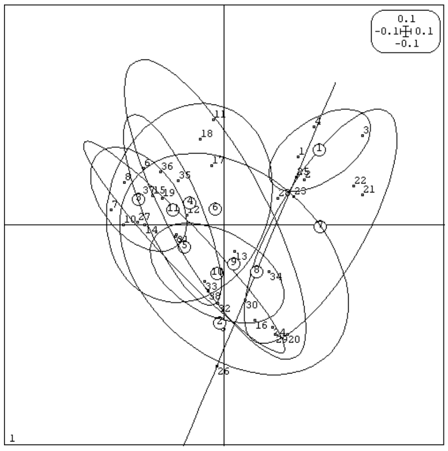

Figure 1. Representation of axis 1 (12% of global variance) and axis 2 (10% of global variance) of PCA on bacterial communities analyzed by A-RISA, for the 11 treatments within the 44 plots at La Bouzule. (1. TEMOIN; 2. LSS; 3. LDSS; 4. LDCSS; 5. LDCSSO; 6. LDCSSM; 7. MPS; 8. CA; 9. HWA; 10. CMF; 11. COF)

No significant difference was observed between treatments when looking at the total microarthropod abundance (Figure 2), total Collembola abundance and total Acari abundance. Furthermore a greater abundance wad observed in the TEMOIN treatment compared to other treatments. No effect was observed on collembolan species richness and diversity.

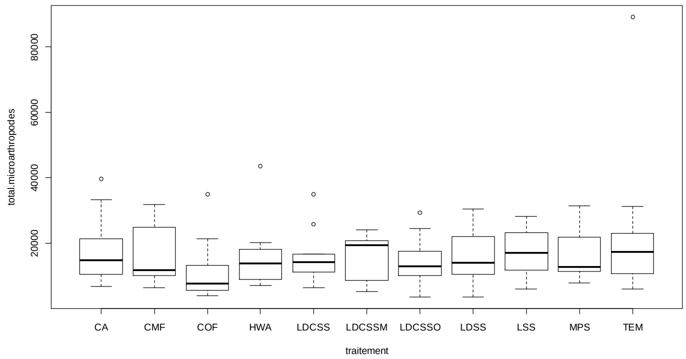

Figure 2. Total abundance of microarthropods in the different treatments

14 orders of macrofauna have been collected on the site (Oligochaeta, Opistophora, Malacostracae, Isopoda, Gastropoda, Arachnida, Chilopoda Lithobiomorpha, Geophilomorpha and Scolopendromorpha, Diplopoda Iulida and Polydesma, Insecta Diptera, Coleoptera, Hymenoptera, Heteroptera and Lepidoptera), with abundance ranging between 444 ind.m-2 in CA to 640 ind.m-in HWA. No significant effect of treatments was observed on macrofauna (abundance, richness and diversity). The structure of functional earthworm communities (epigeic, anecic, endogeic) was similar between treatments, with a dominance of endogeic worms (56% of the total abundance) followed by anecic (36% of the total abundance).
The soil was dominated by the 2-20µm granulodensimetric fraction (40 to 50%); no significant difference between treatments was observed concerning the weight distribution of the granulometric fraction or the typology of microstructures. On the other hand, no trace of sludge residues that had been observed using TEM just after application in the field was significantly observed 8 years later.

Conclusion

Looking at the results obtained on different biological parameters, including Bacteria, mesofauna, macrofauna and organo-mineral associations, it can be concluded that biosolids applied at recommended doses do not have long term effects on soil biological quality.

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