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RESEARCH ARTICLE

Impact of untreated urban waste on the prevalence and antibiotic resistance profiles of human opportunistic pathogens in agricultural soils from Burkina Faso

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Abstract This study examined the long-term effects of the landfill disposal of untreated urban waste for soil fertilization on the prevalence and antibiotic resistance profiles of various human opportunistic pathogens in soils from Burkina Faso. Samples were collected at three sites in the periphery of Ouagadougou during two campaigns in 2008 and 2011. At each site, amendment led to changes in physico-chemical characteristics as shown by the increase in pH, CEC, total C, total N, and metal contents. Similarly, the numbers of total heterotrophic bacteria were higher in the amended fields than in the control ones. No sanitation indicators, i.e., coliforms, Staphylococci, and Enterococci, were detected. *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (Bcc) were detected at a low level in one amended field. *Stenotrophomonas maltophilia* was detected from both campaigns at the three sites in the amended fields and only once in an unamended field. Diversity analysis showed some opportunistic pathogen isolates to be closely related to reference clinical strains responsible for nosocomial- or community-acquired infections in Northern countries. Antibiotic resistance tests showed that *P. aeruginosa* and Bcc isolates had a wild-type phenotype and that most *S. maltophilia* isolates had a multi-drug resistance profile with resistance to 7 to 15 antibiotics. Then we were able to show that amendment led to an increase of some human opportunistic pathogens including multi-drug resistant isolates. Although the application of untreated urban waste increases both soil organic matter content and therefore soil fertility, the consequences of this practice on human health should be considered.

Keywords Human opportunistic pathogen · Soil · Antibiotic resistance · Burkina Faso · Urban waste

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Introduction

Soils on earth are extremely diverse and are characterized by an extreme heterogeneity of physical and chemical properties offering various microsites for microorganism colonization. Therefore, soils are one of the major reservoirs of prokaryotic diversity (Whitman et al. 1998). Human bacterial pathogens can be part of these indigenous soil microbial communities. Some of the species belonging to *Clostridium* (*Clostridium perfringens, Clostridium tetani*, *Clostridium botulinum*), *Bacillus* (*Bacillus anthracis*), *Mycobacterium* (*Mycobacterium tuberculosis*), or *Burkholderia* (*Burkholderia pseudomallei*) genera are considered typical soil inhabitants either because they or their close relatives were isolated from soil samples, i.e., *B. pseudomallei, Bacillus cereus*, and *Mycobacterium bovis* (Young et al. 2005; Kuske et al. 2006; Trung et al. 2011). Similarly, several emergent opportunistic pathogens were reported as abundant in soil and plant rhizospheres. Various species responsible for infections among cystic fibrosis patients (i.e., *Burkholderia cenocepacia, Stenotrophomonas maltophilia,* and species belonging to *Pseudomonas* genus) were frequently isolated from soil and root samples (Berg et al. 2005; Mendes et al. 2013).

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Some of these species (Hickey and Focht 1990; Vedler et al. 2004; Ali et al. 2010) were also frequently isolated from polluted sites such as hydrocarbon- or pesticide-contaminated soils. Pathogens can also be transient inhabitants of soil brought from exogenous sources. Agricultural practices such as irrigation with wastewater, fertilization with municipal waste, or animal-derived products (i.e., bovine manure, pig slurry, poultry droppings) can contribute to the dispersion of both chemical and biological contaminants in terrestrial environments.

Whereas evidence from the impact of toxicants as metals on microbial activity and soil fertility is well documented (Smith 2009; Singh et al. 2011), less is known about the direct or indirect impact of these practices on the dissemination and/or selection of pathogens. Reports from the literature indicated that some pathogens such as *Escherichia coli, Campylobacter jejuni, Yersinia enterolitica,* and *Listeria monocytogenes* can enter the soil through the spread of sewage sludge, municipal solid waste (Garrec et al. 2003; Semenov et al. 2009), and organic waste of animal origin (Meng and Doyle 1997; Farzan et al. 2010). Despite a few reports on the presence of *Pseudomonas aeruginosa* in farmyard manures (Colinon et al. 2013; Deredjian et al. 2014), composted industrial waste (Kaszab et al. 2011), and wastewater treatment plants (Lavenir et al. 2007; Lee et al. 2008; Edrington et al. 2009), little data is available regarding the presence and behavior of opportunistic pathogens in water or solid waste used for agriculture practices in the environment.

Human opportunistic bacteria are known for their antibiotic resistance properties which are traditionally attributed to high intrinsic resistance and the ability to acquire novel resistance genes by horizontal gene transfer (Sanchez et al. 2009; Alvarez-Ortega et al. 2011). The use of antibiotics and antiseptics contributes greatly to the increasing level and subsequent spread of resistance among these species in hospital settings (Canton and Morosini 2011; Livermore 2012). Antibiotic resistance in the environment is also directly affected by the release of antibiotic resistant bacteria and antibiotic residues via waste from human or animals receiving therapeutic antibiotics (Baquero et al. 2008, Jechalke et al. 2014). Manure and biosolids spreading in agricultural lands might be a source of antibiotic resistant bacteria when applied as biofertilizers to farmland as they are known to contain microbial pathogens (Zhu et al. 2013; Su et al. 2015). Consequently, agriculture practices might contribute to the distribution of opportunistic bacteria as well as their resistance properties.

Data from the literature mainly focuses on the distribution of opportunistic pathogens in northern countries as they are of concern near hospitals due to their implication in nosocomial infections and in the colonization of cystic fibrosis patients. Little data is available on the prevalence of pathogens including opportunistic ones in African countries in soils under different pedo-climatic context. Similarly, due to the increase in mineral fertilizer prices, the spreading of municipal waste materials is a common practice among farmers of developing countries in sub-Saharan African areas and especially in urban and peri-urban agriculture (Eaton and Hilhorst 2003; Afon 2007). However, in these areas, waste is often used fields without selection or pre-treatment (Kaboré et al. 2010).

The main objective of this study was to examine the long-term effects of a common agricultural practice, i.e., landfill disposal of untreated urban waste for soil fertilization, on the prevalence of various human opportunistic pathogens in sub-Saharan soils from Burkina Faso with regard to the impact on soil physico-chemical properties. To achieve this, amended and unamended soil samples from three agricultural sites in the periphery of Ouagadougou were sampled during two campaigns in 2008 and 2011. The abundance of culturable bacteria belonging to *P. aeruginosa*, *S. maltophilia* and *Burkholderia cepacia* complex (Bcc) was compared to that of the sanitation indicator bacteria to reveal the impact of exogenous sources on pathogen prevalence. Then within each opportunistic species, both a phylogenetic analysis based on the sequencing of genetic markers and antibiotic resistance profiles were performed to compare African soil isolates to reference strains from international collections of Northern countries.

Material and methods

Field sites and sampling

The study was conducted on three agricultural sites (Tabtenga, Toubwéogo, and Zagtouli) in the periphery of Ouagadougou, Burkina Faso. At each site, local farmers grow sorghum in fields modified every year with untreated solid urban waste (UW) to restore soil fertility. These fertilizers are made of 50–60 % organic household waste (vegetables), as well as paper, glass, plastic, scrap iron, cans, and some contain hospital waste (5 to 20 % of the amendment). The used amount of fertilizers is 20 to 30 t ha^{-1} $year^{-1}$. To evaluate the benefits of soil amendment on agronomic properties and crop production, one nearby field is kept unamended (control field). These sites allow the long-term assessment of the effects of amendment as these ferti individual sites have been used as landfills for between 8 and 20 years. All soils are petroplinthic leptosol (FAO-IUSS-ISRIC 2006). Soil physico-chemical characteristics were measured by the Laboratory of Soil Analysis (INRA Arras, France) using standard methods.

At each site, sampling was carried out at the surface layer (0–5 cm) in an amended field and a control one. In June 2008 (6 months after the crop harvest and about 1 month after amendment), soil samples from each field were collected as follows: three transects per field were done and 10 samples taken every 2 m along a 20-m transect were mixed to make a

composite sample. The 18 samples (3 transects × 2 treatments × 3 sites) were placed in plastic bags and taken to the laboratory, where they were immediately sieved (2-mm mesh size), homogenized, and used for bacterial counts. A second campaign was conducted in February 2011 (3 months after the crop harvest and about 8 months after amendment) on the Tabtenga site. Five samples from one transect were taken from the control field and 25 samples from five transects from the amended field. Each sample is a composite of sub-samples taken every 2 m pooled by two along a 20-m transect. The 30 samples were treated as mentioned above.

Enumeration of TCH, sanitation indicators, and human opportunistic pathogens

Total bacteria were extracted by blending 5 g of soil samples with 50 ml of a 0.8 % (*w*:*v*) sterile NaCl solution for 90 s in a Waring blender (Eberbach Corporation, MI, USA). The homogeneous soil suspension was serially diluted tenfold in sterile saline solution, and 100 μl of appropriate dilutions were spread on the various media. All agars used in this study were purchased from Oxoid (Dardilly, France).

The total culturable heterotrophs (TCH) were enumerated on a tenfold diluted Tryptic Soy Agar medium (TSA1/10). Enumeration of coliforms was carried out on TTC Tergitol 7 media. For Staphylococci and Enterococci recovery, Chapman and Slanetz media were used, respectively.

Regarding opportunistic pathogens, the VIA (vancomycin, imipenem, amphotericin B) selective agar medium was used to detect and isolate *S. maltophilia*. Dark green colonies with a blue halo morphotype were considered to be *S. maltophilia* and counted according to the procedure previously described (Pinot et al. 2011). *P. aeruginosa* isolation was performed using the Cetrimide Agar Base (CAB) medium (Oxoïd) supplemented with nalidixic acid (15 mg l^{-1}). Enrichment assays were performed by transferring 2 g of soil into 20 ml of a salt solution supplemented with acetamide, as described previously (Green et al. 1974). Inoculated enrichment broths were incubated for 3 days at 28 °C with shaking at 180 rpm. All the greenish and yellowish fluorescent colonies were picked from the plates for further identification as described in Lavenir et al. (2007) by targeting *ecf*X. Bcc were enumerated on two selective media: trypan blue tetracycline agar (TB-T) (Hagedorn et al. 1987) and *B. cepacia* selective agar (BCSA) (Henry et al. 1997). Colonies were confirmed as belonging to the Bcc as described in Mahenthiralingam et al. (2000) by targeting *rec*A.

Cycloheximide (200 mg l^{-1}) was added in all media to impair the growth of fungi. Three plates were inoculated per dilution. Bacterial colonies were counted after 5 days of incubation at 28 °C for TSA1/10 plates and after 24 to 48 h of incubation at 37 °C for TTC tergitol 7, Slanetz, and Chapman plates. Plates were incubated at 28 °C for up to 48 h for *S. maltophilia*, at 28 and 37 °C for up to 72 h for *P. aeruginosa*, and at 28 °C for up to 4 days for Bcc.

Sanitation indicator identification and diversity of human opportunistic pathogens

Bacterial DNA was extracted according to Pitcher et al. (1989). To ensure that the target organisms from selective media used for sanitation indicator enumeration were not misidentified, we recorded the number of bacteria in each morphology type and five to ten representatives of each recorded morphology type were selected for further identification based on 16S ribosomal DNA (rDNA) sequencing. 16S ribosomal RNA (rRNA) gene was amplified using the Taq DNA polymerase (MP Biomedicals, CA, USA), with 0.2 μM of 8F and 1492R primers in a 50 μL reaction (Weisburg et al. 1991). Sequencing was performed on the 16S rRNA gene PCR fragment using primers 16S-515F, 16S-906F, and 16S-907R (Weisburg et al. 1991). Sequencing was performed by Biofidal (Villeurbanne, France). Identification to the species level was performed by comparison with the Ribosomal Database Project database (http://rdp.cme.msu.edu/) and by using Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of the identified bacterial isolates were deposited in the GenBank nucleotide sequence database under Accession Numbers KX146470 to KX146483.

Intraspecies diversity among 21 *S. maltophilia* isolates was evaluated using partial *gyr*B gene sequencing as previously described (Yamamoto and Harayama 1998). Additional sequences from previously studied strains in Adamek et al. (2011) and from sequenced genomes (K279, R551-3, RR10, EPM1, SKA14, SO28, PML168, AU12-09, AB55555, D457, JV3) were retrieved from the GenBank database and included in the analysis.

The diversity of 38 *P. aeruginosa* isolates was assessed by the sequencing of the *ecf*X marker as previously described (Lavenir et al. 2007). Fifteen sequences retrieved either from the whole genome of *P. aeruginosa* strains (PAO1, PA1, PA7, PA14, MTB-1, B136-33, PA96, YL84, SCV20265, LES431, DK2, c7447m, NCGM2.S1) or from uncultured environmental bacteria (riv1, lag2) were added.

The diversity analysis of 24 isolates from the Bcc was evaluated by analysis of the phylogeny obtained from the *rec*A sequences. Sequencing and analysis of a 955 bp of the *rec*A gene was performed using the BCR1 and BCR2 primers (Mahenthiralingam et al. 2000). Other *rec*A sequences from representative species belonging to the Bcc were obtained from the EMBL database and added in the *rec*A phylogeny analysis.

Antibiotic resistance test

The in vitro antimicrobial resistances of the isolates were routinely determined using the Vitek®2 system with a card (NO93) dedicated to non-fermenting Gram negative bacteria (Biomérieux, Marcy l'Etoile, France) according to manufacturer instructions. Minimal inhibitory concentrations of 18 antibiotics [ticarcillin (TIC 16, 32, 64 μg/ml), ticarcillin/clavulanic acid (TIM 8/2, 32/2, 64/2 μg/ml), piperacillin (PIP 4, 16, 64 μg/ml), piperacillin/tazobactam (TZP 4/4, 16/4, 128/4 μg/ml), ceftazidim (CAZ 1, 2, 8, 32 μg/ml), cefepim (FEP 2, 8, 16, 32 μg/ml), aztreonam (ATM 2, 8, 32 μg/ml), imipenem (IPM 2, 4, 16 μg/ml), meropenem (MEM 0.5, 4, 16 μg/ml), amikacin (AMK 8, 16, 64 μg/ml), gentamicin (GEN 4, 16, 32 μg/ml), isepamicin (ISP 4, 8, 32 μg/ml), tobramicin (TOB 8, 16, 64 μg/ml), ciprofloxacin (CIP 0.5, 2, 4 μg/ml), pefloxacin (PEF 0.5, 2, 8 μg/ml), colistin (CS 4, 16, 32 μg/ml), minocyclin (MIN 2, 4, 8 μg/ml), and trimethoprim/sulfamethoxazole (SXT 0.5/9.5, 2/38, 16/304 μg/ml)] were determined. MIC results provided by the Vitek®2 system were analyzed by the AES™ (Advanced Expert System) software incorporated in this system. Interpretations were established following the recommendations of the antibiogram committee of the French Society of Microbiology, (http://www.sfm-microbiologie.org).

Statistical analysis

Significant differences in bacterial numbers between soil samples were determined using *R* with Student's *t* test.

The nucleotide sequences obtained in this study and gene sequences previously reported and available in the GenBank database were aligned, and a phylogenetic tree was constructed using the Seaview 4.2 software (Gouy et al. 2010; pbil, Lyon, France). Multiple nucleotide sequence alignment was performed using the MUSCLE alignment algorithm (Edgar 2004). Multiple alignments were then cleaned with Gblocks (Talavera and Castresana 2007). The pairwise evolutionary distances were calculated using the Kimura2-parameter model. A phylogenetic tree was constructed using the neighbor-joining (bioNJ) method with 1000 replicates using bootstrap.

Results

Impact of amendment on soil characteristics

As shown in Table 1, the soil surface layers at the three sites are characterized by a high proportion of sand. At each site, amendment led to changes in soil physico-chemical characteristics as shown by the increase in pH, total C and total N contents, and CEC. Similarly, most metals showed higher values in the amended soils than in the control ones. The

Table 1 Soil physico-chemical characteristics in control field (C) and in field amended with untreated solid urban waste (UW) at each experimental site

	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	Organic matter (g kg^{-1})	Ctot (g kg^{-1})	Ntot (g kg^{-1})	pH_{H2O}	CEC Metson (cmol kg^{-1})	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Hg (mg kg^{-1})
Tabtenga C	78	185	737	5.18	2.99	0.267	5.8	2.21	0.379	39.1	5.65	6.33	10.2	0.007
Tabtenga UW	59	172	769	21.6	12.7	1.29	7.6	4.55	0.559	40.8	14.0	10.9	87.6	0.274
Toubwéogo C	73	314	613	6.69	11.6	0.51	7.1	2.93	0.523	44.0	10.9	14.5	40.3	0.009
Toubwéogo UW	114	263	623	50.8	32.7	2.13	8.1	8.01	0.833	63.5	38.6	117	242	0.071
Zagtouli C	52	218	730	6.52	11.3	0.529	6.6	3.37	0.428	56.7	10.1	10.3	19.0	0.009
Zagtouli UW	107	214	679	41.6	72.0	2.67	8.1	11.2	1.17	80.3	59.5	363	770	0.169

Zagtouli site experienced the highest impact as Cd, Zn, Pb, and Hg were highly enriched in the amended field. On the contrary, the Tabtenga site experienced the least impact as only Hg, and to a lesser extent, Zn, and Cd were higher in the amended soils.

Impact of amendment on TCH and sanitation indicators

Data from the 2008 campaign showed a higher amount of TCH in the amended fields of Tabtenga and Toubwéogo than in the control ones, $p < 0.001$ and $p < 0.01$, respectively (Table 2). No significant differences were observed between the amended and control fields of Zagtouli. The higher amount of TCH in the amended field of Tabtenga was confirmed by the data from the 2011 campaign where the population of TCH was approximately seven times greater in amended fields.

Regarding sanitation indicators, coliforms, Staphylococci, and Enterococci were not isolated from any of the samples of the 2008 campaign whether they were collected in the amended or control fields.

To ensure that sanitation indicators were not misidentified, we selected several colonies per morphotype on the various selective media and identified them based on 16S rDNA sequencing (Table 3). On the TTC tergitol 7 medium (i.e., coliform detection), the yellow colonies and the red ones were all identified as belonging to *Enterobacteriaceae*. They were found to be closely related to *Salmonella enterica*, *Pantoea stewartii*, *Shigella sonnei*, *or Cronobacter helveticus*. The red colonies with a blue halo were found to be closely related to

Table 2 Bacterial counts for total culturable heterotrophs (TSA1/10 medium), sanitation indicators (TTC tergitol 7, Slanetz, and Chapman), and opportunistic pathogens (CAB, VIA, BCSA, and TB-T media) in agricultural soil samples collected at three sites in the periphery of Ouagadougou, Burkina Faso

Site	Number of treated samples	Culturable heterotrophs	Coliforms	Staphylococci	Enterococci	*Pseudomonas aeruginosa*[a]	*Stenotrophomonas maltophilia*		*Burkholderia cepacia complex*	
		TSA1/ 10 CFU ×-10^6	TTC tergitol 7 CFU	Chapman CFU	Slanetz CFU	CAB CFU	Number of positive samples	VIA CFU × 10^2	BCSA CF-U	TB-TC-FU
2008										
Tabtenga C	3	3.92 (±0.166)	0	0	0	0	0	0	nd	nd
Tabtenga UW	3	12.5 (±1.36)	0	0	0	0	1	4.42 (±7.1-5)	nd	nd
Toubwé-ogo C	3	8.63 (±0.529)	0	0	0	0	0	0	nd	nd
Toubwé-ogo UW	3	22.6 (±5.66)	0	0	0	0	1	3.39 (±2.8-9)	nd	nd
Zagtouli C	3	18.0 (±5.29)	0	0	0	0	1	2.97 (±2.5-7)	nd	nd
Zagtouli UW	3	14.5 (±5.66)	0	0	0	0	2	12.1 (±2.0-8)	nd	nd
2011										
Tabtenga C	5	9.67 (±0.519)	nd	nd	nd	0	0	0	0	0
Tabtenga UW	25	55.5 (±4.08)	nd	nd	nd	<10[b]	8	1.03 (±1.5-5)	0	48.6 (±8-.5)[c]

Two campaigns were performed in June 2008 and February 2011. At each site, soil samples were collected in unamended fields (C) and in fields amended with untreated solid urban waste (UW). Counts [colony forming units (CFU) per g drywt sample (± standard deviation)] are either the mean of nine plates for the 2008 campaign or the mean of 75 plates and 15 plates for the amended fields and control ones, respectively, for the 2011 campaign. Standard errors are indicated in brackets

nd not done

[a] These data were partly reported in Deredjian et al. 2014

[b] *P. aeruginosa* was detected in one sample without the enrichment step and in seven samples after the enrichment step

[c] Bcc was detected in 2 out of 25 samples using BCSA and in 10 out of 15 samples on CAB media after the enrichment step

Table 3 16S rDNA-based identification of isolates growing on TTC tergitol 7 or Chapman media

Isolate name	Medium	Size (bp)[a]	Number of isolates with similar sequences	Identification based on sequence producing significant alignment with the maximum score (max identity); accession number
08BF03TD	TTC tergitol 7	1503	5	*Shigella sonnei* SsO46 (98); NR_074894
08BF07TK	TTC tergitol 7	1440	3	*Burkholderia phenoliruptrix* BR3459a (99); NR_102849
08BF11TN	TTC tergitol 7	1451	3	*Pantoea stewartii* subp. *Indologenes* CIP104006 (98); NR_104928
08BF19TK	TTC tergitol 7	1505	2	*Massilia albidiflava* 45 (98); NR-043,308
08BF31TG	TTC tergitol 7	1483	6	*Ralstonia mannitolilytica* LMG6866 (99) ; NR_025385
08BF31TL	TTC tergitol 7	1533	2	*Salmonella enterica* subsp. *Enterica* serovar Typhi Ty2 (98); NR_074799
08BF25TD	TTC tergitol 7	1493	14	*Cupriavidus plantarum* ASC-64 (99); NR_109160
08BF05TG	TTC tergitol 7	1412	3	*Cronobacter helveticus* LMG23732 (99); NR_104980
08BF01CA	Chapman	1463	13	*Bacillus licheniformis* DSM13 (99); NR_118996
08BF03CD	Chapman	1514	4	*Bacillus endophyticus* 2DT (99); NR_025122
08BF27CA	Chapman	1433	8	*Bacillus aryabhattai* B8W22 (100); NR_115953
08BF15CA	Chapman	1456	10	*Bacillus subtilis* subsp. *Subtilis* OS-6.2 (99); NR_114996
08BF29CG	Chapman	1463	5	*Bacillus firmus* NBRC 15306 (99); NR_112635
08BF27CB	Chapman	1439	11	*Bacillus cereus* CM 2010 (99); NR_115714

[a] Length of the consensus sequence among isolates showing a similar sequence

species belonging to β-Proteobacteria, i.e., *Cupriavidus plantarum*, *Massilia abidiflava*, *Ralstonia solanacearum*, or *Burkholderia phenoliruptrix*. Fifty-one colonies selected on the Chapman media (i.e., Staphylococci detection) showed homology with *Bacillus* sequences such as *Bacillus licheniformis*, *Bacillus endophyticus, Bacillus aryabhattai, Bacillus subtilis, Bacillus firmus* or *B. cereus*. No colonies were observed on the Slanetz plates (i.e., Enterococci detection).

Impact of amendment on opportunistic pathogens

S. maltophilia was not detected in the control fields of Toubwéogo and Tabtenga in the 2008 samples. However, it was detected in the control field of Zagtouli and in the amended fields of all three sites. At each site, the highest values were detected in the amended fields. *S. maltophilia* was only detected in the amended field in the 2011 samples from Tabtenga. The numbers of *S. maltophilia* varied from 103 (±155) to 1210 (±208) CFU (g drywt soil)$^{-1}$. The *S. maltophilia* population represented between 0.0002 % (amended field of Tabtenga 2011) and 0.008 % (amended field of Zagtouli 2008) of the total heterotrophs. It has to be noted that the number of *S. maltophilia* colonies on plates was always below ten and that variations were observed between samples from the same field, as some samples led to the detection of *S. maltophilia* whereas most of the others did not.

As reported earlier (Deredjian et al. 2014), no *P. aeruginosa* was detected in the control and amended soils from the 2008 campaign among all sites. None of the 30 samples from the 2011 sampling campaign at the Tabtenga site except one from the amended field site led to a direct isolation of *P. aeruginosa*. This sample was analyzed three times and either zero or one colony was obtained. The estimation of *P. aeruginosa* abundance was below 10 CFU g^{-1} dry soil. The enrichment assays led to the detection of *P. aeruginosa* in 7 out of 25 samples collected in the amended field.

Samples from the 2011 campaign were also analyzed for the presence of Bcc isolates because we reported that isolates closely related to Bcc were isolated on the CAB media from amended soil samples collected in 2008. Two different media were then used. Surprisingly, no Bcc isolate was obtained on the TB-T media. Only 2 out of 25 samples led to the detection of Bcc on the BCSA media whereas 10 out of 15 led to the detection on the CAB media after the enrichment step. Therefore, the estimated amount of Bcc in the amended field of Tabtenga was 48.6 (±8.5) CFU (g drywt soil)$^{-1}$.

Genetic diversity within *S. Maltophilia*, *P. aeruginosa*, and Bcc

Partial sequencing of *gyrB* showed that the 21 *S. maltophilia* isolates recovered from the three sites in 2008 (n = 14) and from the Tabtenga site in 2011 (n = 7) belong to various clusters (Fig. 1). Strains from the 2008 campaign clustered either with the clinical strains K279, Ab5555, and DSM50170 or with the strain LMG11114. Each cluster contained isolates from two or three sites, suggesting the presence of a strain in several sites. None of the isolates from the 2011 campaign at the Tabtenga site clustered with K279a. Three of them, i.e., MEEB16, MEEB18, and MEEB20, were

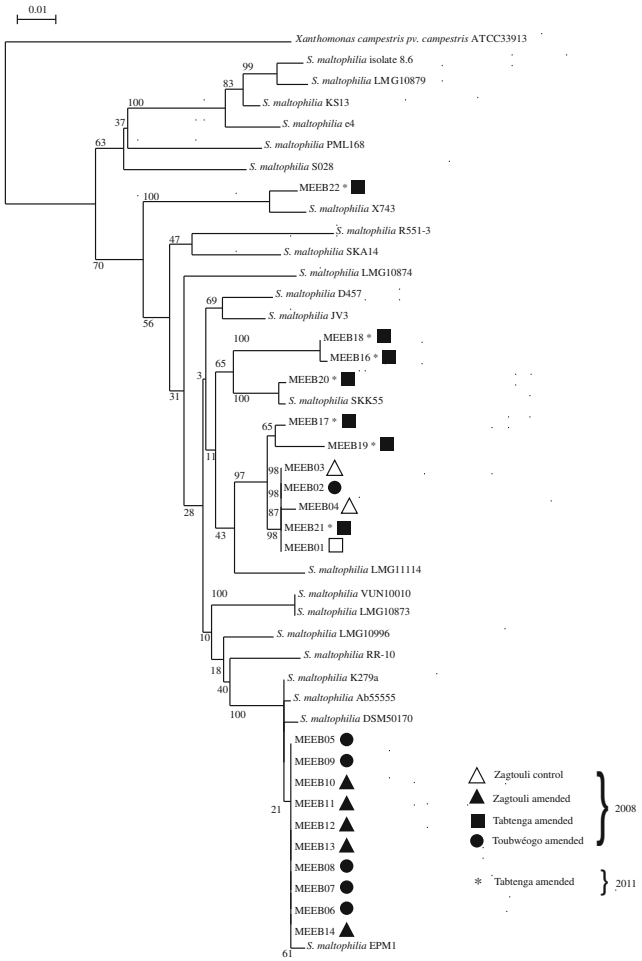

Fig. 1 Neighbor-joining tree based on partial *gyr*B gene sequences. Neighbor-joining tree of 21 *S. maltophilia* isolates and international reference strains with xanthomonads used as outgroups

closely related to LMG11114. The others, i.e., strains MEEB17, MEEB19, and MEEB21 and strain MEEB22, were closely related either to SKK55 or to X743, respectively.

Sequencing of *ecfX* gene was performed to estimate the relation between the 38 isolates of *P. aeruginosa* recovered from the amended Tabtenga field (four isolates from the direct isolation and 34 from the enrichment procedure) with *P. aeruginosa* from other geographical regions and from clinical origin. Data showed that strains from Burkina Faso clustered in two major groups (Fig. 2). Nine isolates clustered between the outlier strain PA7 and all other Burkina Faso isolates and collection strains. Seventeen isolates shared the same sequence as EML1262 and clustered with most of the reference strains (PA01, PA1, PA96, YL84, LES431, DK2, c7447m, SCV20265). The remaining isolates including EML1263, EML1264, and 11BFE3335 clustered with the other reference strains PA14, B136-33, MTB1, and NCGM2.S1. A previous study (Youenou et al. 2014) on the typing of *P. aeruginosa* isolates from soils including seven isolates from the present study (EML1258 to EML1264) had shown an important diversity despite the low number of isolates analyzed and their common soil origin (Tabtenga site) and that none of them had a multi-locus variable number tamdem repeat analysis (MLVA) profile similar to the other clinical and environmental strains studied.

Sequencing of the *recA* gene and phylogeny analysis showed that Bcc isolates from the Tabtenga site (22 from the CAB media in 2008 and two recovered from the BCSA media in 2011) were closely related to *B. cenocepacia* (two isolates), *Burkholderia latens* (one isolate), *Burkholderia diffusa* (one isolate), *Burkholderia dolosa* (17 isolates), and *Burkholderia multivorans* (three isolates) (Fig. 3). Clustering did not allow the differentiation of the isolates whether they were obtained from the CAB isolation procedure or the BCSA media and whether they were recovered from the 2008 or the 2011 campaigns.

Antibiotic resistance properties of soil opportunistic pathogens

Antimicrobial resistance properties of 38 *P. aeruginosa* isolates, 21 *S. maltophilia* isolates, and 24 Bcc isolates were tested. Like the wild type (WT), we previously showed that all *P. aeruginosa* isolates (100 %) were resistant to minocycline and trimethoprim/sulfamethoxazole and sensitive to colistin, ciprofloxacin, isepamicin, amikacin, meropenem, aztreonam, cefepim, and ceftazidim (Youenou et al. 2014).

In the present study, we showed the isolates belonging to the Bcc have a profile similar to the wild type of *B. cepacia*. They were all resistant to ticarcilline, ticarcillin/clavulanic acid, quinolones and aminosides, imipenem, and colistin and sensitive to the other antibiotics.

All isolates of *S. maltophilia* from the 2008 campaign from both control and amended fields resist to at least ten antibiotics (Fig. 4) and were considered multi-resistant as they were able to resist to antibiotics belonging to at least three different classes. These resistances encompass penicillins, cephalosporins, monobactam, and carbapenems including meropenem, aminoglycosides, and polymixin. Some multi-drug resistant isolates showed increased resistances compared to strain K279a, the clinical antibiotic resistant reference strain, as they also showed resistance to fluoroquinolones. These multi-drug resistant isolates were detected at the three sites in amended and/or control fields. The genetic mechanisms underlying the resistance to these antibiotics was investigated for two of these isolates, i.e., strains MEEB01 and MEEB05 previously named BurA1 and BurE1, using comparative genomics and reported in Youenou et al. (2015). On the contrary, the isolates from the Tabtenga site sampled in 2011 showed differential susceptibilities with resistance to 3 to 13 antibiotics out of the 15 tested (Fig. 4).

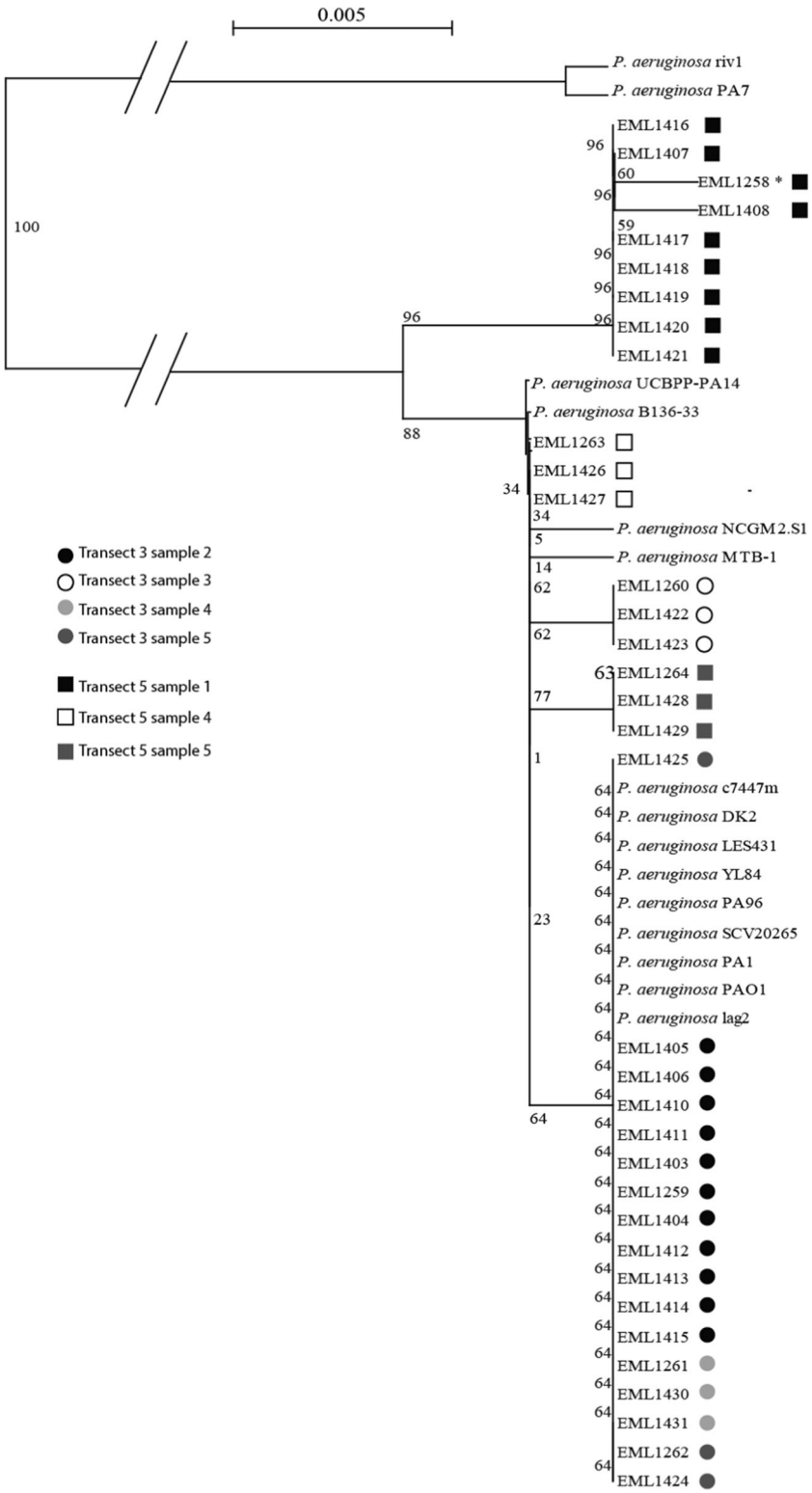

Fig. 2 Neighbor-joining tree based on total *ecf*X gene sequences from 38 *P. aeruginosa* isolates from the 2011 campaign at the Tabtenga site and various *P. aeruginosa* reference strains. *Asterisk* indicates the sequence of four isolates obtained without an enrichment step

Discussion

Spreading of urban waste is widely used in African regions to increase soil fertility and therefore crop production. However, spreading without selection or pre-treatment could lead to important sanitary and environmental risks, as this practice is increasingly used in sub-urban agriculture to satisfy the food demand of a rising population. As shown at the three studied sites, this practice impacted the physico-chemical properties of soil. We observed that not only organic matter but also pH

Fig. 3 Neighbor-joining tree based on partial *rec*A gene sequences from 24 *Bcc* isolates obtained from the Tabtenga site and various reference strains. Sequence from *B. pseudomallei* strain K96243 was used as the outgroup. All isolates were recovered from CAB (cetrimide agar base) plate after an acetamide enrichment step except two isolates (*) recovered on BCSA (*Burkholderia cepacia* selective agar) plate. ¤ indicates the two strains isolated from the 2008 sampling campaign

and metal (mercury, zinc, lead, or cadmium) concentrations increased in amended fields. The heavy metals may be due to batteries, disposable household materials (i.e., bottle tops), and plastics in the untreated waste. The bioavailability of metals is known to be strongly influenced by pH and organic matter contents. Whether changes in these parameters affect microbial diversity and functions in these soils need to be investigated further.

Our data showed that amendment led to an increase in total culturable heterotrophs as well as an increase of bacteria able to grow on the various selective media used (data not shown). Similarly, at each site, the amount of opportunistic pathogens was higher in amended fields than in control ones. These observations are probably related to the repeated addition of organic matter over years. The addition of organic matter in agricultural soils represents a readily available source of energy, carbon, and nutrients for soil microorganisms. An increase in C-biomass and a stimulation of soil enzymatic activities have been reported (Albiach et al. 2000). Municipal solid waste-compost amendment generally increases the fungal and bacteria populations in amended soils (Crecchio et al. 2001; Debosz et al. 2002; Smith 2009). As soils from our study are very poor in organic matter, the addition of fresh organic material (vegetable residues) might be an easy-to-use carbon source that enhances the level of heterotrophic bacteria (Bastida et al. 2008). Waste addition also led to an increase in crop production. Consequently, this plant effect might indirectly favor microbial growth including the opportunistic pathogens as these species, i.e., *S. maltophilia* and Bcc are well known as rhizospheric bacteria (Berg et al. 2005).

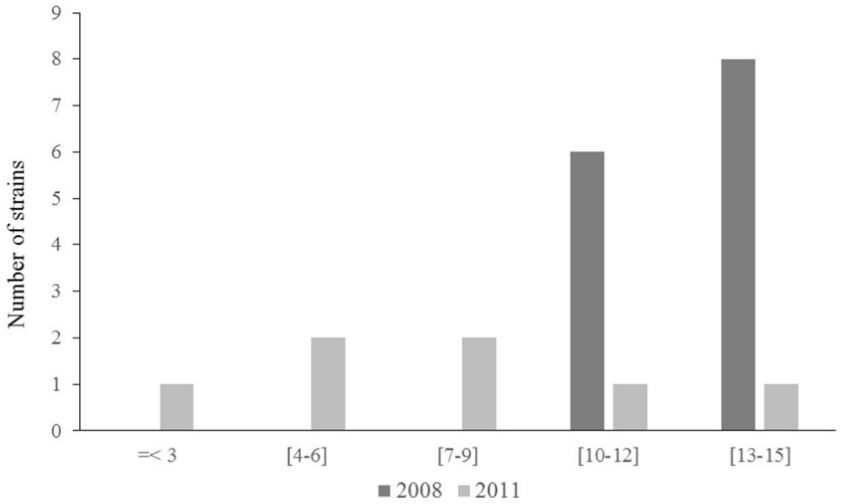

Fig. 4 Antibiotic resistance of seven *S. maltophilia* isolates from the 2011 campaign and 14 *S. maltophilia* isolates from the 2008 sampling campaign. *Brackets* = number of antibiotics

S. maltophilia has often been isolated from plants including rice (Hardoim et al. 2012), potato (Garbeva et al. 2001), and sweet flag (Marecik et al. 2008). The presence of Bcc in soil environments and especially in the rhizosphere of Graminae plants is well documented (i.e., Balandreau et al. 2001; Ramette et al. 2005; Dalmastri et al. 2007). The observed increase in heterotrophic bacteria and two out of the three opportunistic pathogen species, i.e., *S. maltophilia* and Bcc, can then be a consequence of the growth of indigenous soil populations. These pathogens might be present in the control soils but at a level below the detection limit of our methodologies. Amendment would favor their enrichment as it increased the amount of total heterotrophic bacteria. Our study focused on the bulk soil and showed that *S. maltophilia* and Bcc are able to survive in bulk soil despite the lack of plants and thus the low amount of nutrient supply. As these species are known to be abundant in the rhizosphere, it would then be interesting to evaluate how sorghum modifies the abundance and diversity of these species.

The increase in heterotrophs and pathogens could also be partly and/or temporarily due to the addition and growth of exogenous populations brought through amendment. As the waste used in the studied sites are of multiple origins, i.e., domestic, hospital (plasma pockets were seen during the time of sampling, Nazaret personal communication), and are not treated to eliminate pathogens, the latter could be disseminated in soils. Our data from the 2008 campaign showed the lack of detection of coliforms, Staphylococci, and Enterococci, suggesting that either these populations were absent from the initial waste, and/or that they do not persist at a detectable level using our methodology. We expected to detect some of these species given that untreated waste can contain potential pathogenic bacteria such as *Salmonella* or *E. coli* in a higher density than treated amendments, and fecal coliforms and streptococci, which indicate fecal contamination, frequently reach a density of 10^7–10^8 CFU (g drywt soil)$^{-1}$ (around 10^3 in treated composts) (Deportes et al. 1998; Hassen et al. 2001). However, it has to be noted that detection using the selective media used in this study was not as efficient as it could have been since several non-targeted species were able to grow and hampered the true estimation of the sanitary indicators.

Regarding opportunistic pathogens, *P. aeruginosa* is known to be present in animal- and human-originating waste (Lavenir et al. 2007; Deredjian et al. 2014). We were not able to detect *P. aeruginosa* following the 2008 campaign but we did detect that species in some samples from the 2011 campaign. This was probably due to the analysis of a greater number of samples (Deredjian et al. 2014). Also, its sporadic detection in the amended soil might be due to its presence in the urban waste. However, both our field campaigns were conducted during the high and dry seasons. As *P. aeruginosa* is known to prefer wet and humid conditions (Selezska et al. 2012; Deredjian et al. 2014), it would then be interesting to sample and analyze soil samples collected during the rainy season and also less distantly from the time of amendment spreading.

We analyzed the diversity among the various isolates from each pathogen species and compared them to reference strains from internal collections including clinical strains. Among *P. aeruginosa* isolates, we found two major groups with one group containing only strains from Burkina Faso. This observation suggested that the sub-Saharan soils harbor not only the common clones detected worldwide but also specific populations. Our previous study on the genetic typing of *P. aeruginosa* isolates using the MLVA approach showed on a subset of the isolate collection from Burkina Faso that intra-species diversity exists at a low spatial scale (Youenou et al. 2014) and that these isolates do not share common profiles with all other included strains. A more discriminant method such as MLST should then be used to better relate these strains from Burkina Faso to the global *P. aeruginosa* population and evaluate whether some of the well-defined clones and clonal

complexes (i.e., clone C, PA14, PA7) are present in African soils. The *gyrB* analysis revealed a low diversity among *S. maltophilia* isolates compared to what the literature reports from an epidemiological study. However, using a more discriminant genotyping method, i.e., pulse field gel electrophoresis (PFGE), we showed that most of the isolates from the 2008 campaign had a unique profile. The 14 isolates grouped in 11 PFGE profiles (data not shown) showing the high diversity within *S. maltophilia* populations recovered from a narrow geographical area. Interestingly, isolates of the same *gyrB* or PFGE genotypes were observed in the various sites, indicating the dissemination and/or the presence of common indigenous populations in various soils of the periphery of Ouagadougou. Regarding Bcc, the *rec*A analysis showed that various species common among cystic fibrosis patients were present in the soils. *B. cenocepacia* genomovar IIIA-, *B. dolosa*-, and *B. multivorans*-like strains were identified. It has to be noted that few environmental isolates for these three species are available despite the fact that all these species have been frequently encountered among cystic fibrosis individuals and other clinical infections (Mahenthiralingam et al. 2000; Holden et al. 2009). To our knowledge, this is the first report of *B. dolosa* detection in soil. *B. cenocepacia* genomovar IIIA and *B. multivorans* were detected in the rhizosphere of maize in the USA (Ramette et al. 2005) as well as in the rhizosphere of maize and wheat in France and Australia (Balandreau et al. 2001). *B. ambifaria*-like strains were not detected in our soils despite their frequent detection and at a high level in the rhizosphere of various plants (Coenye et al. 2001; Dalmastri et al. 2007).

Urban waste may not only bring pathogens but also contaminants that could have selected for antibiotic resistance. For instance, contaminants such as the metals detected in the amended soils could favor the emergence of antibiotic resistance as these compounds could lead to both metal and antibiotic resistance (Baker-Austin et al. 2006). Our data on antibiotic resistance properties showed that our *P. aeruginosa* isolates had a wild phenotype suggesting that these isolates, whether they originated from waste or soils, were not exposed to a selective pressure favoring the development of resistance. Similarly, the Bcc isolates exhibited no additional resistance to the intrinsic ones these species might have. These observations would suggest that metals were not available in the soil or that their levels were not high enough to lead to antibiotic resistance. On the contrary, *S. maltophilia* isolates were mostly found to be resistant to several antibiotics. This observation did not come as a surprise as this species is characterized by a high level of intrinsic resistance to a variety of structurally unrelated antimicrobials (Sanchez et al. 2009) and is able to acquire new genes through gene transfer (Hu et al. 2011). Antibiotic hydrolysis or modification, target gene modification, membrane permeability alteration, and efflux pump overexpression are among the various mechanisms enabling *S. maltophilia* to resist antimicrobials. Based on the literature, including our previous work, environmental isolates are reported to be as resistant as clinical ones (Berg et al. 1999; Deredjian et al. 2016) and to harbor as many resistance genes (Sanchez et al. 2009). The data from the present study therefore confirms these reports in terms of antibiotic resistance phenotypes. Similarly, whole genome sequencing of two strains recovered from the studied sites and comparative genomics confirmed that environmental strains have as many and diverse antibiotic resistant genes as clinical strains have (Youenou et al. 2015). Further studies are then needed to elucidate the origin of the multi-resistant phenotype and the role of environmental conditions in this multi-resistance selection.

In summary, spreading untreated urban waste on agricultural fields is a matter of great concern as it enhances both the amount of toxicants, i.e., metals, and directly or indirectly affects the amount of opportunistic pathogens closely related to the clones or clonal complexes involved in nosocomial- or community-acquired infections including multi-drug resistant isolates in soils. We could then strongly suggest performing an appropriate pre-treatment of these wastes before their use for agricultural practices. The pre-treatment should include separation of organic materials from others and be adapted for the reduction of both chemical and biological contaminants. While our study only focused on the selection pressure of metals, future studies should also include antimicrobials as we witnessed the presence of leftover pharmaceuticals and plasma packets in the amended fields during the various sampling campaigns.

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