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Isolation and identification of fungal communities in compost and vermicompost

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Abstract: This research illustrates the qualitative and quantitative composition of the mycoflora of both a green compost (thermophilically produced from plant debris) and a vermicompost (mesophilically produced by the action of earthworms on plant and animal wastes after thermophilic preconditioning). Fungi were isolated using three media (PDA, CMC, PDA plus cycloheximide), incubated at three temperatures (24, 37 and 45 C). Substantial quali-quantitative differences in the species composition of the two composts were observed. The total fungal load was up to 8.2×10^5 CFU/g dwt in compost and 4.0×10^5 CFU/g dwt in vermicompost. A total of 194 entities were isolated: 118 from green compost, 142 from vermicompost; 66 were common to both. Structural characterization of this kind is necessary to determine the most appropriate application of a compost and its hygienic quality.

Key words: compost, compost hygiene, compost quality, earthworms, fungi

INTRODUCTION

Composting is the biological conversion of solid organic waste into usable end products such as fertilizers, substrates for mushroom production and biogas. Moreover, their high organic matter content and biological activity make composts effective in a variety of applications, including erosion control, revegetation, biofiltration and bioremediation (Alexander 1999).

The active component involved in the biodegradation and conversion processes during composting is the resident microbial community, among which fungi play a very important role. The biomass ratio of fungi to prokaryotes in compost is about 2:1 (Sparling et al 1982, Wiegant 1992). In addition, fungi use many carbon sources, mainly lignocellulosic polymers and can survive in extreme conditions. They mainly are responsible for compost maturation (Miller 1996).

A better understanding of fungal diversity in compost may prove crucial in predicting its best application. Fungi affect soil fertility, suppress plant diseases and promote mushroom growth (Straatsma and Samson 1993). They also degrade complex polymers such as polyaromatic compounds or plastics and are being increasingly applied to bioremediate soils contaminated with a wide range of pollutants (Kastner and Mahro 1996, Eggen and Sveum 1999, Minussi et al 2001). Monitoring fungal diversity is essential to detect fungi hazardous to humans, animals and plants and to optimize compost quality standards (Summerbell et al 1994).

Much information exists about the succession of fungi, mainly thermotolerant and thermophilic fungi, in conventional two-phase thermogenic composting (Straatsma et al 1994, Ross and Harris 1983, Fermor et al 1979, Chang and Hudson 1967). These data refer mainly to mushroom compost, straw compost or experimental compost obtained by environmentally controlled and standardized processes. However, industrial composting uses a variety of procedures and raw materials (Beffa et al 1998) and hence results in very different end products.

In contrast, very little is known about fungal communities in mesophilic processes such as vermicomposting, an alternative technology increasingly used in many countries, including Italy (Beffa et al 1998, Masciandaro et al 2000). Earthworms stabilize organic residues and reduce pathogenic bacteria and other human pathogens (Eastman et al 2001) and also can greatly affect fungal communities. They select fungal species by influencing spore germination and creating microsites favorable or unfavorable to fungus development (Brown 1995, Tiunov and Scheu 2000). The few studies on these mechanisms have provided partly contradictory data and stressed the importance of monitoring the hygienic aspects of this mesophilic process in fungal communities (Beffa et al 1998).

In brief, since composting methods and different source materials are associated with differences in the composition of a fungal community, monitoring of the resident fungal population in a compost is

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needed to determine its quality and field of application (Peters et al 2000). This work focuses on the species composition and load of the mycoflora of two mature composts marketed by an Italian firm: a compost currently used as a bioactivator in landfills and a vermicompost mainly applied in agriculture.

MATERIALS AND METHODS

The following physical dimensions, temperatures and moisture measurements were taken outdoors and are approximations, unless denoted otherwise. Compost (C) was produced in an outdoor pile (3 m wide, 50 m long, 1.5 m high) from plant debris from various sources by a conventional thermophilic process that lasted ca 6 mo, during which the piles were turned periodically by machine. The maximum temperature during the composting was 60 C. The pH of the final product was 7.2, the moisture content 40%, humic organic carbon (humic acid + fulvic acid) 3.6% of the dry matter and the C/N ratio 15. Vermicompost (VC) was produced in an outdoor pile (3 m wide, 50 m long, 0.5 m high) composed of 70% dung (from cows, poultry and various zoo animals) and 30% plant debris from various sources. After preconditioning for several days, during which the temperature rose to 60 C, earthworms (*Lumbricus rubellus* Hoffmeister) were added (50×10^3 worms per m^3 organic matter) and the pile was turned periodically by machine. During this mesophilic phase the temperature never exceeded 25–30 C. The pH of the final product was 7.9, the moisture content 38.5%, humic organic carbon (humic acid + fulvic acid) 7.6% of the dry matter and the C/N ratio about 25. Both composts were stored in polypropylene bags 1–3 mo at 10 C before being sold.

Ten approximately 1 kg samples per compost ($C_{1–10}$, $VC_{1–10}$), were examined according to the guidelines proposed by the Piedmont Region (Trombetta et al 1998). A 10 g portion of each sample was suspended in 90 ml $Na_4P_2O_7 \cdot 10\ H_2O$ to disperse organic colloids; further dilutions were made in NaCl (0.9%). The final dilution (1: 20 000) was plated (1 ml per plate) on 11 replicates: five of potato-dextrose agar (PDA), three of carboxy-methyl cellulose agar (CMC) and three of PDA supplemented with cycloheximide (CX) to retard the growth of all fungi, allow isolation of slow-growing colonies and focus on fungi of medical interest (Airaudi and Filipello Marchisio 1996, Filipello Marchisio et al 1996). Plates were incubated at 24 C, 37 C and 45 C to isolate mesophilic and thermotolerant/thermophilic fungi with the result that 33 replicates were made for each sample. The number of colony forming units per g of dry weight (CFU/g dwt) was calculated both for the total mycoflora and for each species or morphotype.

Fungi were identified conventionally according to their macroscopic and microscopic features. After determination of their genera (Domsch et al 1980, von Arx 1981, Hanlin 1990, Kiffer and Morelet 1997), they were transferred to the media recommended by the authors of selected genus monographs for species identification. Sterile mycelia (SM) were classified according to their hyphal pigments and their production of chlamydospores, sclerotia or vesicles. SM with clamp connections or positive to the reaction with Diazonium Blue B salts (DBB), according to Summerbell (1985), were classified as basidiomycetes.

The nonparametric Mann-Whitney test for independent groups (StatView 1988) was run to assess the significance ($P \leq 0.05$) of the differences between the two composts (total load, species and genera load) and between all treatments (three media and three incubation temperatures) in the composts. Diversity indexes based on species richness (Margalef index) and species relative abundance (Berger-Parker, Shannon, Simpson indexes) were applied to assess biodiversity (Biodiversity PRO 1997). According to Magurran (1988), the Margalef index was calculated from the formula $D_{Mg} = (S - 1)/\ln N$ (here and throughout, S is the number of fungal entities and N is the total number of individuals); the Berger-Parker index from the formula $d = N_{max}/N$ (where N_{max} is the number of individuals in the most abundant species); the Shannon index from the formula $H' = -Sp_i(\ln p_i)$ (where p_i is the proportion of individuals found in the ith species); the Simpson index from the formula $D = \Sigma\{[n_i(n_i - 1)]/[N(N - 1)]\}$. As diversity increases, d and D decrease. We therefore used these indexes in their reciprocal form 1/d and 1/D (Magurran 1988). The Mann-Whitney test (StatView 1988) was run to assess the significance ($P \leq 0.05$) of the differences of each index between the two composts. Moreover, the two population structures were analyzed with the rank-abundance plot (Biodiversity PRO 1997). Multivariate analysis (Detrended Correspondence Analysis-DCA) was used to evaluate quali-quantitative differences in the composition of the mycofloras of the two composts and between the 10 samples of each compost (CANOCO 1998). All statistics were obtained from the highest load of each species in the 9 treatments of each sample.

RESULTS

The total fungal load was high in both composts: from 5.0×10^4 to 8.2×10^5 CFU/g dwt in C, and from 5.3×10^4 to 4.0×10^5 CFU/g dwt in VC, depending on media or incubation temperature (TABLE I). The culture and/or incubation conditions produced different load values within the same compost. In C, a significant reduction in CFU/g dwt was induced by higher incubation temperatures and the addition of cycloheximide. In VC, higher temperatures, cycloheximide and carboxy-methyl cellulose all reduced CFU/g dwt values. The load values in C always were higher, except for CMC at 45 C and CX at all temperatures (TABLE I).

A total of 194 fungal entities were identified from the two composts, of which 118 came from C and 142 from VC. Only 66 were common to both composts (TABLE II). The greatest number of species were isolated from both composts on PDA incubated at 24 C. Employment of the CMC and CX media, however, and incubation at 37 C and 45 C allowed

TABLE I. Mean fungal load (CFU/g dwt ± SE) and number of fungal entities isolated in compost (C) and vermicompost (VC) on 3 media (PDA, CMC, CX) incubated at 24 C, 37 C, 45 C. Number of entities isolated from C only and from VC only in brackets

	C	No. of fungal entities	VC	No. of fungal entities
PDA 24 C	$8.2{\cdot}10^5 \pm 1.6{\cdot}10^5$ a*	70 (31)	$4.0{\cdot}10^5 \pm 8.0{\cdot}10^4$ a*	98 (46)
PDA 37 C	$8.1{\cdot}10^5 \pm 1.6{\cdot}10^5$ a*	42 (6)	$2.4{\cdot}10^5 \pm 4.0{\cdot}10^4$ a*	52 (11)
PDA 45 C	$3.8{\cdot}10^5 \pm 1.1{\cdot}10^5$ b*	43 (7)	$1.2{\cdot}10^5 \pm 3.1{\cdot}10^4$ b*	37 (3)
CMC 24 C	$6.4{\cdot}10^5 \pm 1.5{\cdot}10^5$ a*	32 (5)	$1.5{\cdot}10^5 \pm 4.9{\cdot}10^4$ c*	30 (4)
CMC 37 C	$4.6{\cdot}10^5 \pm 9.5{\cdot}10^4$ ab*	17 (3)	$1.5{\cdot}10^5 \pm 4.1{\cdot}10^4$ ac*	19 (4)
CMC 45 C	$2.8{\cdot}10^5 \pm 9.8{\cdot}10^4$ bc	9 (0)	$9.0{\cdot}10^4 \pm 2.9{\cdot}10^4$ bc	15 (1)
CX 24 C	$2.3{\cdot}10^5 \pm 6.9{\cdot}10^4$ d	25 (3)	$2.3{\cdot}10^5 \pm 1.0{\cdot}10^5$ c	30 (2)
CX 37 C	$1.4{\cdot}10^5 \pm 5.6{\cdot}10^4$ d	5 (0)	$7.5{\cdot}10^4 \pm 2.7{\cdot}10^4$ c	19 (4)
CX 45 C	$5.0{\cdot}10^4 \pm 2.1{\cdot}10^4$ c	3 (0)	$5.3{\cdot}10^4 \pm 2.0{\cdot}10^4$ bc	6 (1)

Different letters indicate significant differences ($P \leq 0.05$, Mann-Whitney test) among the load of the same compost obtained in different culture condition and/or incubation temperatures and * indicates significant differences between C and VC in the same culture conditions.

the isolation of a good number of species that otherwise would have been missed: 24 from C and 30 from VC (TABLE I).

In VC, the greater number of species corresponds to higher biodiversity index values (TABLE III). The lower evenness of C is illustrated in the rank abundance plot (FIG. 1), which demonstrates the quantitative domination of two species, namely the *Scedosporium* state of *Pseudallescheria boydii* and *Aspergillus fumigatus*.

The DCA scatterplot (FIG. 2) shows the distribution of the samples and the 194 fungal entities. There are three zones along the 1 axis: zone I containing most of the C samples ($C_{4–10}$) and the entities found only or preponderant in C; zone III containing most of the VC samples ($VC_{1, 2, 4–7}$) and the entities found only or preponderant in VC; zone II containing C and VC samples ($C_{1–3}$ e $VC_{3, 8–10}$) and the entities equally distributed between C and VC, or found only in C or in VC, but present in smaller quantities and thus regarded as occasionals (FIG. 2).

The 194 fungal entities comprised 117 mitosporic fungi, 45 ascomycetes, 15 zygomycetes, 14 SM morphotypes and three basidiomycete morphotypes (TABLE II). Both composts were dominated by mitosporic fungi (including the ascomycetes in their anamorphic state) (TABLE IV). Of the most abundant species in both composts, the thermotolerant fungus *Scedosporium* state of *Pseudallescheria boydii* displayed a significantly greater load ($P = 0.0012$) in C (7.3×10^5 CFU/g dwt) and was associated with it in the DCA and included in zone I (FIG. 2). The genera with the highest load and number of species in both composts were *Penicillium* and *Aspergillus*. The total load of *Penicillium* was 3.0×10^5 CFU/g dwt in VC and 1.2×10^5 CFU/g dwt in C. This difference is not significant, though *P. aurantiogriseum* var. *aurantiogriseum* ($P = 0.013$) and *P. roseopurpureum* ($P = 0.03$) showed prevalence in C and associated with C_8 and C_5 respectively (FIG. 2), and many species solely were present in C or in VC (TABLE II). The *Aspergillus* load was not significantly different: 1.8×10^5 CFU/g dwt in C and $1.3.10^5$ CFU/g dwt in VC. Both loads were composed mainly of thermotolerant *A. fumigatus* var. *fumigatus* (TABLE II), which displayed high loads in all the samples and was located in zone II (FIG. 2).

Other species described as thermotolerant or thermophilic (Domsch et al 1980) were isolated at 37 and 45 C from both composts with no significant load differences. They included *Aspergillus fumigatus* var. *ellipticus, Malbranchea cinnamomea, Paecilomyces variotii* and *Thermomyces lanuginosus. Absidia corymbifera* alone was isolated from C only.

There were no significant differences between composts in the quantitative composition of the two sets of *Cladosporium* and *Acremonium* species (about 5.0×10^4 and 1.0×10^4 CFU/g dwt respectively in both composts), whereas *Fusarium* species prevailed in C (3.1×10^4 in C versus 2.1×10^3 CFU/g dwt in VC), mainly in C_4 (FIG. 2), and *Trichoderma* species (8.2×10^3 CFU/g dwt) were present exclusively in *C. Chrysosporium* and *Scopulariopsis* species prevailed in VC (respectively 1.2×10^4 in VC versus 0 CFU/g dwt in C, and 3.1×10^4 in VC versus 1.7×10^4 CFU/g dwt in C). The number of species and the load of ascomycetes were higher in VC (TABLE IV), particularly owing to the presence of *Corynascus sepedonium* (mainly present in VC_3), *Eurotium chevalieri* (mainly present in VC_6), and *Talaromyces flavus* var. *flavus* (mainly present in $VC_{2,10}$) (TABLE II, FIG. 2).

The load of zygomycetes was similar in C and VC with greater species diversity in C (TABLE IV), mainly

TABLE II. Fungal entities isolated from compost (C) and vermicompost (VC) and their load (CFU/g dwt) expressed as the average of the highest values recorded in each of the 10 samples

	C CFU/g dwt	VC CFU/g dwt
1 *Absidia corymbifera* (Cohn) Saccardo & A. Trotter	$4.3 \cdot 10^2$	—
2 *Acremonium charticola* (Lindau) W. Gams	$1.7 \cdot 10^3$	—
3 *Acremonium chrysogenum* (Thrirumalachar & Sukapure) W. Gams	$4.3 \cdot 10^2$	$1.9 \cdot 10^3$
4 *Acremonium fusidioides* (Nicot) W. Gams	$1.1 \cdot 10^3$	—
5 *Acremonium humicola* (Onions & Barron) W. Gams	$6.8 \cdot 10^2$	—
6 *Acremonium persicinum* (Nicot) W. Gams	$1.4 \cdot 10^3$	$9.0 \cdot 10^2$
7 *Acremonium sclerotigenum* (F. & V. Moreau ex Valenta) W. Gams	$1.7 \cdot 10^3$	$5.0 \cdot 10^3$
8 *Acremonium* sp. 1	$4.5 \cdot 10^2$	$9.0 \cdot 10^2$
9 *Acremonium* sp. 2	—	$3.0 \cdot 10^2$
10 *Acremonium strictum* W. Gams	$3.7 \cdot 10^3$	$7.0 \cdot 10^2$
11 *Acrodontium griseum* (Fassatiovà) de Hoog	$9.5 \cdot 10^2$	
12 *Acrophialophora fusispora* (S.B. Saksena) Samson	$1.8 \cdot 10^3$	$1.8 \cdot 10^3$
13 *Alternaria alternata* (Fries: Fries) von Keissler	$2.2 \cdot 10^3$	$9.0 \cdot 10^2$
14 *Aphanoascus terreus* (Randhawa & Sandhu) Apinis (*Chrysosporium* state)	—	$1.0 \cdot 10^3$
15 *Apiospora montanei* Saccardo (*Arthrinium* state)	—	$9.0 \cdot 10^2$
16 *Arthroderma tuberculatum* Kuehn (*Myceliophthora* state)	—	$1.0 \cdot 10^3$
17 *Ascodesmis microscopica* (Crouan) Seaver	$6.7 \cdot 10^2$	—
18 *Aspergillus candidus* Link : Fries	—	$2.0 \cdot 10^3$
19 *Aspergillus flavus* Link : Fries var. *flavus*	$1.2 \cdot 10^3$	—
20 *Aspergillus flavus* Raper & Fennell var. *columnaris*	$5.5 \cdot 10^3$	$3.9 \cdot 10^3$
21 *Aspergillus fumigatus* Fresenius var. *fumigatus*	$1.2 \cdot 10^5$	$1.1 \cdot 10^5$
22 *Aspergillus fumigatus* Raper & Fennel var. *ellipticus*	$5.4 \cdot 10^3$	$1.0 \cdot 10^4$
23 *Aspergillus niger* van Tiegham	$1.1 \cdot 10^4$	$9.0 \cdot 10^2$
24 *Aspergillus ochraceus* Wilhelm	$1.2 \cdot 10^3$	$3.0 \cdot 10^2$
25 *Aspergillus oryzae* (Ahlburg) Cohn var. *oryzae*	$7.3\ 10^2$	—
26 *Aspergillus puniceus* Kwon & Fennell	—	$9.0 \cdot 10^2$
27 *Aspergillus sulphureus* (Fresenius) Thom & Church	—	$8.0 \cdot 10^2$
28 *Aspergillus terreus* Fennel and Raper var. *africanus*	$7.3 \cdot 10^2$	
29 *Aspergillus terreus* Thom var. *terreus*	$4.1 \cdot 10^3$	$3.3 \cdot 10^3$
30 *Aspergillus versicolor* (Vuillemin) Tiraboschi	$2.7 \cdot 10^4$	$6.6 \cdot 10^3$
31 *Aspergillus wentii* Wehmer	$6.8 \cdot 10^2$	—
32 *Aureobasidium pullulans* (de Bary) Arnaud var. *pullulans*	—	$9.0 \cdot 10^2$
33 *Beauveria bassiana* (Balsamo) Vuillemin	—	$4.0 \cdot 10^3$
34 *Beauveria brongniartii* (Saccardo) Petch	—	$2.0 \cdot 10^3$
35 *Botryotinia fuckeliana* (de Bary) Whetzel (*Botrytis* state)	—	$2.3 \cdot 10^3$
36 *Chaetomium bostrycodes* Zopf	$9.0 \cdot 10^2$	—
37 *Chaetomium funicola* Cooke	—	$3.0 \cdot 10^2$
38 *Chaetomium globosum* Kunze : Fries	—	$2.9 \cdot 10^3$
39 *Chaetomium nigricolor* L. Ames	$4.3 \cdot 10^2$	—
40 *Chrysosporium indicum* (Randhawa & Sandhu) Garg	—	$4.7 \cdot 10^3$
41 *Chrysosporium merdarium* (Link : Fries) Carmichael	—	$1.0 \cdot 10^3$
42 *Chrysosporium queenslandicum* Apinis & Rees	—	$3.0 \cdot 10^2$
43 *Chrysosporium tropicum* Carmichael	—	$6.0 \cdot 10^3$
44 *Cladosporium chlorocephalum* (Fresenius) Mason & M.B. Ellis	$2.2 \cdot 10^3$	—
45 *Cladosporium cladosporioides* (Fresenisus) de Vries	$3.6 \cdot 10^4$	$3.2 \cdot 10^4$
46 *Cladosporium herbarum* (Persoon : Fries) Link	$1.0 \cdot 10^4$	$5.5 \cdot 10^3$
47 *Cladosporium oxysporum* Berkeley & Curtis	$4.3 \cdot 10^2$	$2.0 \cdot 10^3$
48 *Cladosporium sphaerospermum* Penzig	$3.9 \cdot 10^3$	$9.4 \cdot 10^3$
49 *Cordyceps memorabilis* Cesati (*Paecilomyces* state)	$1.0 \cdot 10^3$	—
50 *Corynascus sepedonium* (C.W. Emmons) von Arx	—	$6.7 \cdot 10^3$
51 *Corynascus sepedonium* (C.W. Emmons) von Arx (*Myceliophthora* state)	$6.0 \cdot 10^3$	—
52 *Cunninghamella elegans* Lendner	—	$1.5 \cdot 10^4$
53 *Cylindrocarpon* sp.	$9.0 \cdot 10^2$	—

TABLE II. Continued

		C CFU/g dwt	VC CFU/g dwt
54	*Doratomyces microsporus* (Saccardo) Morton & G. Smith	$1.1 \cdot 10^3$	—
55	*Doratomyces purpureofuscus* (Schweinitz : Fries) Morton & G. Smith	—	$9.0 \cdot 10^2$
56	*Emericella nidulans* (Eidam) Vuillemin var. *nidulans*	—	$7.0 \cdot 10^2$
57	*Engyodontium album* (Limber) de Hoog	$1.4 \cdot 10^3$	$8.0 \cdot 10^2$
58	*Epicoccum nigrum* Link	$2.9 \cdot 10^3$	$9.0 \cdot 10^2$
59	*Eremascus fertilis* Stoppel	$1.8 \cdot 10^3$	—
60	*Eurotium amstelodami* Mangin	$4.5 \cdot 10^2$	$2.7 \cdot 10^3$
61	*Eurotium chevalieri* Mangin	—	$6.6 \cdot 10^3$
62	*Eurotium intermedium* Blaser	—	$1.1 \cdot 10^3$
63	*Eurotium montevidense* (Talice & Mackinnon) Malloch & Cain	$6.8 \cdot 10^2$	—
64	*Eurotium rubrum* Konig et al	—	$8.0 \cdot 10^2$
65	*Eutypella scoparia* (Schweinitz : Fries) Ellis & Everhart (*Libertella* state)	$6.8 \cdot 10^2$	—
66	*Exophiala moniliae* de Hoog	$7.3 \cdot 10^2$	—
67	*Exophiala pisciphila* McGinnis & Padhye	—	$9.0 \cdot 10^2$
68	*Exophiala* sp.	—	$1.0 \cdot 10^3$
69	*Fennelia nivea* (Wiley & Simmons) Samson (*Aspergillus* state)	—	$1.0 \cdot 10^3$
70	*Fusarium oxysporum* Schlechtendahl : Fries	$4.7 \cdot 10^2$	—
71	*Fusarium* sp. 1	$5.8 \cdot 10^3$	—
72	*Fusarium* sp. 2	$1.9 \cdot 10^4$	—
73	*Fusarium* sp. 3	—	$9.0 \cdot 10^2$
74	*Fusarium* sp. 4	$2.0 \cdot 10^3$	—
75	*Geomyces pannorum* (Link) Sigler & Carmichael var. *pannorum*	$1.4 \cdot 10^4$ a	$10.0 \cdot 10^3$ b
76	*Geotrichum* sp.	—	$1.0 \cdot 10^3$
77	*Gilmaniella macrospora* Moustafa	—	$7.0 \cdot 10^2$
78	*Gliocladium* sp.	$2.4 \cdot 10^3$	—
79	*Graphium putredinis* (Corda) S. Huges	—	$1.0 \cdot 10^3$
80	*Haematonectria haematococca* (Berkeley & Broome) Samuels & Niremberg (*Fusarium* state)	$3.7.10^3$ a	$9.0 \cdot 10^2$ b
81	*Humicola fuscoatra* Traaen var. *fuscoatra*	$9.9 \cdot 10^3$	$3.0 \cdot 10^3$
82	*Humicola grisea* Cooney & Emerson var. *thermoidea*	—	$9.0 \cdot 10^2$
83	*Hypocrea rufa* (Person : Fries) Fries (*Trichoderma* state)	$4.7 \cdot 10^3$	—
84	*Leptographium* sp.	$4.1 \cdot 10^3$	$6.0 \cdot 10^3$
85	*Leptosphaeria coniothyrium* (Fuckel) Saccardo (*Coniothyrium* state)	$4.3 \cdot 10^2$	$1.8 \cdot 10^3$
86	*Malbranchea cinnamomea* (Libert) van Oorschot & de Hoog	$2.4 \cdot 10^4$	$1.1 \cdot 10^4$
87	*Microascus brevicaulis* S.P. Abbott (*Scopulariopsis* state)	—	$6.0 \cdot 10^3$
88	*Microascus cirrosus* Curzi	—	$1.0 \cdot 10^3$
89	*Microascus manginii* (Loubière) Curzi (*Scopulariopsis* state)	$6.2 \cdot 10^3$	$1.0 \cdot 10^3$
90	*Moniliella suaveolens* (Burri & Staub) de Hoog var. *nigra*	—	$2.9 \cdot 10^4$
91	*Mortierella alliacea* Linnemann	$2.3 \cdot 10^3$	—
92	*Mortierrella alpina* Peyronel	$8.9 \cdot 10^2$	$6.0 \cdot 10^3$
93	*Mortierella chlamydospora* (Chesters) van der Plaats-Niterink	$4.5 \cdot 10^2$	—
94	*Mortierella echinosphaera* van der Plaats-Niterink	$3.6 \cdot 10^4$	$1.1 \cdot 10^3$
95	*Mortierella globalpina* W. Gams & Veen baas-Rijks	$6.7 \cdot 10^2$	—
96	*Mortierella humilis* Linnemann ex W. Gams	$3.7 \cdot 10^3$	—
97	*Mortierella hyalina* (Harz) W. Gams	$7.1 \cdot 10^3$	—
98	*Mortierella indohii* C.Y. Chien	$2.0 \cdot 10^3$	—
99	*Mortierella* sp. 1	$1.7 \cdot 10^4$	$2.3 \cdot 10^4$
100	*Mortierella* sp. 2	$7.9 \cdot 10^{33}$	$1.0 \cdot 10^4$
101	*Mortierella* sp. 3	—	$4.7 \cdot 10^3$
102	*Mucor circinelloides* (Hagem) Schipper f. *griseo-cyanus*	—	$3.0 \cdot 10^2$
103	*Nectria* sp.	$6.8 \cdot 10^2$	—
104	*Neosartorya fischeri* (Wehemer) Malloch & Cain var. *fischeri*	—	$3.0 \cdot 10^3$
105	*Neosartorya spinosa* (Raper & Fennell) Kozakiewicz	—	$7.0 \cdot 10^2$
106	*Paecilomyces variotii* Bainier	$1.8 \cdot 10^3$	$4.5 \cdot 10^3$

TABLE II. Continued

	C CFU/g dwt	VC CFU/g dwt
107 *Penicillium aurantiogriseum* Dierckx var. *aurantiogriseum*	$1.9{\cdot}10^4$ a	$3.0{\cdot}10^2$ b
108 *Penicillium brevicompactum* Dierckx	$2.0{\cdot}10^4$	$1.5{\cdot}10^5$
109 *Penicillium canescens* Sopp	$1.5{\cdot}10^3$	—
110 *Penicillium chermesinum* Biourge	—	$2.0{\cdot}10^3$
111 *Penicillium chrysogenum* Thom	$2.0{\cdot}10^3$	$1.3{\cdot}10^4$
112 *Penicillium citrinum* Thom	$2.4{\cdot}10^3$	$1.0{\cdot}10^4$
113 *Penicillium dierckxii* Biourge	$4.8{\cdot}10^2$	—
114 *Penicillium digitatum* Saccardo	$1.4{\cdot}10^3$	$2.0{\cdot}10^3$
115 *Penicillium diversum* Raper & Fennell	$1.4{\cdot}10^3$	—
116 *Penicillium echinulatum* Raper & Thom ex Fassatiovà var. *echinulatum*	$1.2{\cdot}10^3$	$4.7{\cdot}10^3$
117 *Penicillium expansum* Link	$4.8{\cdot}10^2$	—
118 *Penicillium glabrum* (Wehmer) Westling	$8.1{\cdot}10^3$	$1.1{\cdot}10^4$
119 *Penicillium glandicola* (Oudemans) Seifert & Samson	—	$3.0{\cdot}10^2$
120 *Penicillium herquei* Bainier & Sartory	$4.3{\cdot}10^2$	$3.0{\cdot}10^4$
121 *Penicillium implicatum* Biourge	$4.7{\cdot}10^2$	$2.0{\cdot}10^3$
122 *Penicillium islandicum* Sopp	—	$1.0{\cdot}10^3$
123 *Penicillium italicum* Wehmer var. *italicum*	—	$9.0{\cdot}10^2$
124 *Penicillium janczewskii* Zaleski	$2.1{\cdot}10^3$	$9.0{\cdot}10^2$
125 *Penicillium jensenii* Zaleski	$1.1{\cdot}10^3$	$2.0{\cdot}10^3$
126 *Penicillium minioluteum* Dierckx	$7.3{\cdot}10^2$	—
127 *Penicillium ochrochloron* Biourge	$5.1{\cdot}10^2$	—
128 *Penicillium paxilli* Bainer	$1.5{\cdot}10^3$	—
129 *Penicillium piceum* Raper & Fennel	—	$7.8{\cdot}10^3$
130 *Penicillium purpurescens* (Sopp) Raper & Thom	—	$2.0{\cdot}10^3$
131 *Penicillium purpurogenum* Stoll	$4.5{\cdot}10^3$	$8.0{\cdot}10^2$
132 *Penicillium restrictum* Gilmann & Abbott	$6.4{\cdot}10^3$	$1.7{\cdot}10^3$
133 *Penicillium rolfsii* Thom var. *rolfsii*	$4.5{\cdot}10^2$	—
134 *Penicillium roquefortii* Thom	$1.4{\cdot}10^3$	—
135 *Penicillium roseopurpureum* Dierckx	$2.8{\cdot}10^4$ a	$1.2{\cdot}10^3$ b
136 *Penicillium rugulosum* Thom	—	$9.0{\cdot}10^2$
137 *Penicillium simplicissimum* (Oudemans) Thom	$7.9{\cdot}10^2$	$3.9{\cdot}10^3$
138 *Penicillium* sp. 1	—	$3.5{\cdot}10^4$
139 *Penicillium spinulosum* Thom	—	$9.0{\cdot}10^2$
140 *Penicillium verrucosum* Dierckx var. *verrucosum*	$8.3{\cdot}10^3$	—
141 *Penicillium waksmanii* Zaleski	$1.7{\cdot}10^3$	$1.2{\cdot}10^4$
142 *Phialemonium obovatum* W. Gams & McGinnis	$9.1{\cdot}10^2$	—
143 *Phialophora cyclaminis* van Beyma	—	$2.3{\cdot}10^3$
144 *Phialophora hoffmannii* group (van Beyma) Schol-Schwarz	—	$9.0{\cdot}10^2$
145 *Phialophora* sp.	$7.1{\cdot}10^2$	$3.0{\cdot}10^3$
146 *Phoma exigua* Desmaziéres var. *exigua*	—	$9.0{\cdot}10^2$
147 *Phoma* sp.	—	$2.0{\cdot}10^3$
148 *Phomopsis* sp.	$4.3{\cdot}10^2$	$6.0{\cdot}10^3$
149 *Plectosporium tabacinum* (van Beyma) M.E. Palm, W. Gams & Niremberg (*Fusarium* state)	—	$3.0{\cdot}10^2$
150 *Preussia fleischhakii* (Auerswald) Cain	—	$1.2{\cdot}10^3$
151 *Preussia* sp.	—	$3.0{\cdot}10^2$
152 *Pseudallescheria boydii* (Shear) McGinnis et al	—	$1.7{\cdot}10^3$
153 *Pseudallescheria boydii* (Shear) McGinnis et al (*Scedosporium* state)	$7.3{\cdot}10^5$ a	$1.2{\cdot}10^5$ b
154 *Pseudogymnoascus roseus* Raillo (*Geomyces* state)	$7.3{\cdot}10^2$	—
155 *Rhizopus oryzae* Went & Prinsen Geerligs	$6.8{\cdot}10^2$	—
156 *Rollandina capitata* Patouillard	$1.3{\cdot}10^3$	—
157 *Scopulariopsis brumptii* Salvanet-Duval	$7.9{\cdot}10^2$	$3.9{\cdot}10^3$
158 *Scopulariopsis koningii* (Oudemans) Vuillemin	$1.0{\cdot}10^4$	$1.7{\cdot}10^4$
159 *Scopulariopsis sphaerospora* Zach	—	$3.0{\cdot}10^3$

TABLE II. Continued

		C CFU/g dwt	VC CFU/g dwt
160	*Scytalidium lignicola* Pesante	$7.1 \cdot 10^2$	—
161	*Stachybotrys chartarum* (Ehrenberg) S. Hughes	—	$1.9 \cdot 10^3$
162	*Staphylotrichum coccosporum* J.A. Meyer & Nicot	$1.9 \cdot 10^3$	$9.0 \cdot 10^2$
163	*Syncephalastrum racemosum* Cohn ex Schroter	—	$1.0 \cdot 10^3$
164	*Talaromyces flavus* (Klocker) Stolk & Samson var. *flavus*	—	$8.5 \cdot 10^3$
165	*Talaromyces helicus* (Raper & Fennell) C.R. Benjamin var. *helicus*	—	$1.8 \cdot 10^3$
166	*Talaromyces helicus* Stolk & Samson var. *major*	$4.9 \cdot 10^3$	$7.0 \cdot 10^2$
167	*Thermomyces lanuginosus* Tsiklinsky	$9.3 \cdot 10^2$	$1.8 \cdot 10^4$
168	*Thielavia basicola* Zopf	—	$7.0 \cdot 10^2$
169	*Thielavia heterothallica* von Klopotek (*Myceliophthora* state)	—	$4.7 \cdot 10^3$
170	*Thysanophora penicilloides* (Roumeguère) Kendrick	—	$9.0 \cdot 10^2$
171	*Torrubiella confragosa* Mains (*Verticillium* state)	$1.3 \cdot 10^4$	$7.0 \cdot 10^2$
172	*Trichoderma hamatum* (Bonorden) Bainier	$4.8 \cdot 10^2$	—
173	*Trichoderma harzianum* Rifai	$3.0 \cdot 10^3$	—
174	*Trichosporiella sporotrichoides* van Oorschot	—	$9.0 \cdot 10^2$
175	*Ulocladium alternariae* (Cooke) Simmons	$6.8 \cdot 10^2$	—
176	*Verticillium nigrescens* Pethybridge	—	$1.6 \cdot 10^3$
177	*Westerdykella dispersa* (Clum) Cejp & Milko	—	$7.0 \cdot 10^2$
178	Basidiomycetes with clamp connections	—	$1.9 \cdot 10^3$
197	Basidiomycetes DBB+	$9.7 \cdot 10^3$	$6.0 \cdot 10^3$
180	Basidiomycetes with arthroconidia DBB+	$1.4 \cdot 10^3$	$8.4 \cdot 10^3$
181	Avellanea sterile mycelia	—	$5.1 \cdot 10^3$
182	Dark sterile mycelia	$1.5 \cdot 10^4$	$2.5 \cdot 10^4$
183	Dark sterile mycelia with chlamydospores	—	$4.2 \cdot 10^3$
184	Dark sterile mycelia with chlamydospores in chain	—	$7.8 \cdot 10^3$
185	Dark sterile mycelia with chlamydospores in chain and setole	$6.7 \cdot 10^2$	$2.0 \cdot 10^3$
186	Dark sterile mycelia with red exudate	—	$7.0 \cdot 10^2$
187	Dark sterile mycelia with sclerotia	$1.5 \cdot 10^3$	$7.0 \cdot 10^2$
188	Dark sterile mycelia with setole	—	$9.0 \cdot 10^2$
189	Dark sterile mycelia with vesicles	$1.2 \cdot 10^4$	$4.6 \cdot 10^4$
190	Hyaline sterile mycelia	$2.0 \cdot 10^4$	$1.8 \cdot 10^4$
191	Hyaline sterile mycelia with vesicles	—	$9.0 \cdot 10^3$
192	Hyaline sterile mycelia with chlamydospores	—	$1.6 \cdot 10^3$
193	Yellow sterile mycelia	$4.4 \cdot 10^4$	$9.0 \cdot 10^2$
194	Yellow sterile mycelia with red reverse	—	$7.0 \cdot 10^2$

Different letters indicate significant differences ($P < 0.05$, Mann-Whitney test) among the load of the same species in compost and vermicompost.

due to the genus *Mortierella* (10 species versus 5) (TABLE II), whose species fall mainly in zone I (FIG. 2). *Rhizopus oryzae* and *Absidia corymbifera* were present only in C_5 and C_3 respectively, whereas *Cunninghamella elegans* was present only in VC_{8-10} and *Mucor circinelloides* f. *griseocyanus* in VC_2 (TABLE II, FIG. 2).

Few basidiomycete morphotypes were isolated (2 from C, 3 from VC) compared with the SM morphotypes (6 from C, 14 from VC) (TABLE IV). Dark SM were more varied in morphology in VC (mainly traceable in zone III) and overall load was 3× higher (8.7×10^4 CFU/g dwt in VC versus 2.9×10^4 CFU/g dwt in C) (TABLE II).

DISCUSSION

These results contribute to the microbiological understanding of commercial composts, whose fungal component is often overlooked despite the favorable and unfavorable effects of fungi in the situations in which composts are employed.

C, made mainly from plant debris, displayed a fungal load up to 8.2×10^5 CFU/g dwt. This load is comparable with that observed in the richest soils (Thorn 1997) and justifies the use of C as a bioactivator in landfills. In VC the load was almost halved (up to 4.0×10^5 CFU/g dwt), though still very high

TABLE III. Diversity indices (Margalef, Berger-Parker, Shannon, Simpson indeces) of fungal communities in compost (C) and vermicompost (VC)

	C	VC
Margalef D_{Mg}	18.73 ± 0.77 a	23.01 ± 0.79 b
Berger-Parker 1/d	2.39 ± 0.97 a	3.84 ± 1.86 b
Shannon H′	0.87 ± 0.18 a	1.08 ± 0.24 b
Simpson 1/D	4.28 ± 2.29 a	8.56 ± 4.84 b

Different letters indicate significant differences ($P \leq 0.05$, Mann-Whitney test) between the values of diversity indices in C and VC.

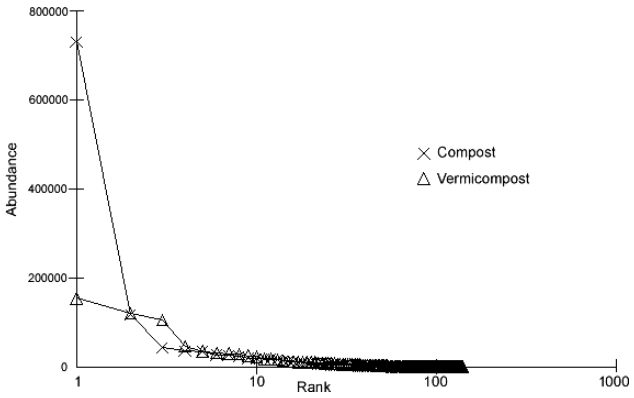

FIG. 1. Rank abundance plot of compost (cross) and vermicompost (triangle) fungal communities. Abundance is the fungal load expressed as CFU/g dwt.

and greater than in many agricultural soils (Luppi Mosca et al 1976).

Employment of a conventional isolation technique results in the identification in both composts of a huge number of species compared with similar studies (Straatsma et al 1994, Fermor et al 1979, Cailleux 1973). This was due to the use of three kinds of media and three incubation temperatures to increase the chances of isolating rare or less competitive species.

Rapid molecular PCR-based techniques now are used to overcome the problems with cultivation-based, time-consuming techniques that allow only investigation of the cultivable portion of the mycoflora and cannot provide a precise quantitative estimate. However, molecular methods identify most bacteria, but only identify a few fungus species in samples from complex environments such as composts, as demonstrated by Roberts and collaborators (2002) in a study of an in-vessel compost and by Peters and collaborators (2000) in a study of composting of agricultural substrates. The main obstacles stem from inefficient DNA extraction, non-optimal primer selection, incompleteness of gene databases and low taxonomic resolution of DNA sequences (Anderson et al 2003, Bridge et al 2003, VanderGheynst et al 2002, Peters et al 2000, Smit et al 1999). In our opinion, molecular techniques only complement the conventional techniques that remain indispensable for the complete study of fungus communities and provide pure cultures that can be used for further physiological characterization of each isolate.

The lower fungal density observed in VC is accompanied by a wider biodiversity. All diversity indexes, in fact, were significantly higher in VC, showing both a greater species richness (Margalef index) and a greater evenness (Berger-Parker, Shannon, Simpson indexes), the latter also shown by the rank abundance plot. The higher biodiversity may be due to a favorable action of earthworms (Brown 1995, Tiunov and Scheu 2000), or to a more varied composition of the raw materials and to the mesophilic conditions prevalent during vermicomposting that are conducive to more types of fungi. The differences in the qualitative and quantitative composition of the mycoflora in C and VC are well represented in the DCA plot. Most C and VC samples are distinguished in function of the presence of species regarded as typical of each matrix because they are present, either exclusively or preponderantly. The DCA, however, also shows that some samples of both composts cannot be separated because they are composed of a similar mycoflora.

Most of the 66 species common to both composts belong to the *Acremonium, Aspergillus, Cladosporium, Malbranchea, Penicillium, Pseudallescheria* and *Thermomyces* genera, many regarded as the most common in composting materials, due to their thermotolerance and/or capacity to degrade a wide range of organic waste (Miller 1996).

Several thermotolerant or thermophilic species (Domsch et al 1980) were isolated from both composts. Their overall load was about 9×10^5 CFU/g dwt in C and about ⅓ in VC. This substantial load, produced by a mesophilic process in a compost, might accumulate because thermophilic preconditioning could encourage the development and proliferation of thermotolerant or thermophilic species; species that can survive during the preparation and life of the finished product.

Among the more abundant species in both composts, we found *Scedosporium* state of *Pseudallescheria boydii* and *Aspergillus fumigatus*. This finding is of particular interest because both species are potential human and animal pathogens. Moreover, we found a substantial presence in VC of *Chrysosporium* and *Scopulariopsis* species, which frequently demonstrate keratinolytic activity (Filipello Marchisio et al 1986, 1991, 1994a, b; Filipello Marchisio 2000), enabling them to invade and parasitize cornified tissues (Rip-

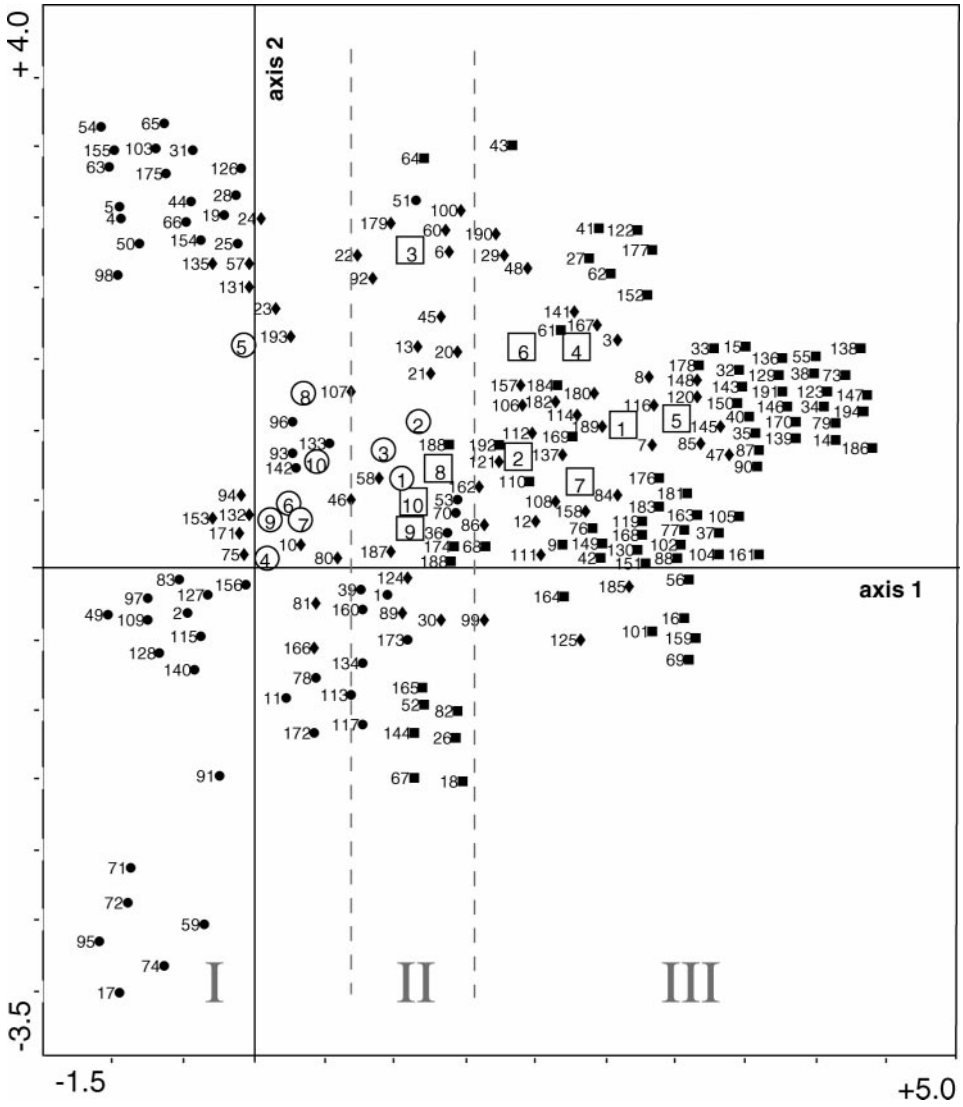

FIG. 2. Scatterplot of the DCA of 10 samples of compost (circle) and 10 samples of vermicompost (square) along with 194 fungal entities (for species name refer to TABLE II). The first two axes are shown (eigenvalues: axis 1 = 0.576; axis 2 = 0.297). ● = species exclusive of compost, ■ = species exclusive of vermicompost, ♦ = species common to both composts.

TABLE IV. Number of fungal genera and species among different taxonomic groups in compost (C) and vermicompost (VC) and their relative load (%)

	C			VC		
	No. of genera	No. of species or morphotypes	Load %	No. of genera	No. of species or morphotypes	Load %
Zygomycetes	3	12	6	3	7	6
Ascomycetes	7	9	1	12	20	4
Basidiomycetes	—	2	1	—	3	2
Mitosporic fungi (including Ascomycetes in their anamorphic state)	37	89	86	44	98	76
Sterile mycelia	—	6	6	—	14	12

pon 1982, Odds 1991). This result contrasts data of Tiunov and Scheu (2000), who found the quantitative and qualitative abundance of *Chrysosporium* species affected detrimentally through earthworms' digestion. The extent earthworms influence the development of health-threatening fungi, however, can be determined only by comparing identically composed raw materials. Since our vermicompost contained animal wastes, the presence of animal skin, hairs and nails would provide a ready explanation for the greater development of these keratinolytic species. These data show the importance of monitoring fungi in compost in order to evaluate its hygienic quality and to establish recommendations on the management of compost by workers and users.

Ascomycetes and to a lesser degree, basidiomycetes, were more abundant and more varied in vermicompost. This too, could be caused by different composition of the two composts, or to preferential grazing by earthworms on fast-growing fungi (such as zygomycetes and mitosporic fungi), rendering them less competitive and conferring an advantage for slower growing K-selected fungi (basidiomycetes and some ascomycetes) (Moody et al 1992). Gut passage stress and the establishment of unfavorable microniches in the compost following the direct and indirect action of earthworms also would explain why the perfect states of *Pseudallescheria boydii* and *Corynascus sepedonium* were found only in VC.

Sterile (particularly dematiaceous) mycelia prevailed in VC as previously demonstrated by Beffa and collaborators (1998). The relationship between earthworms and dematiaceous fungi is uncertain. There is some evidence that they prefer these fungi (Shaw 1992, Marfenina and Ischenko 1997, Beffa et al 1998, Maraun et al 1998). Other workers, however, maintain that their ingestion is impeded by protective chemical barriers, namely the melanin in their hyphal walls (Dash et al 1984, Striganova et al 1988). Zygomycetes diversity (especially *Mortierella* spp.) was lower in VC, as already observed by Brown (1995) and Tiunov and Scheu (2000).

Another point is the isolation of a low number of potentially phytopathogenic species from both composts, particularly VC with its significantly lower *Fusarium* load. These data are supported by the absence of phytotoxicity in these composts shown by the results of seed germination, root elongation and vegetative tests (shoot and root dry weight, shoot height and other growth parameters) (Caccavo 2002). Widespread application of these composts as fertilizers can be recommended.

Along with the systematic characterization of fungal communities in compost, a functional analysis is needed to highlight potentials and applications. Preliminary results show that taxonomic fungal diversity reflects a different metabolic potential (Anastasi et al 2004). Moreover, several fungal strains from these composts now are being investigated to test their capability to decolorize several synthetic dyes and degrade some polycyclic aromatic hydrocarbons: naphthalene, pyrene and benzo (ghi)perylene in microcosms in order to elucidate their potential application in bioremediation.

This research demonstrates that qualitative and quantitative characterization of a compost's fungal community is an essential first step for indicating the best fields of application, and for preparation of quality certificates and correct management practices to safeguard the health of compost workers and users.

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