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Storage method affects disease suppression of flax wilt induced by composts

E. van Rijn*, A.J. Termorshuizen, A.H.C. van Bruggen

Wageningen University, Plant Sciences, Biological Farming Systems, Marijkeweg 22, 6709 PG Wageningen, The Netherlands

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Abstract

Compost can have a disease suppressive effect, but compost research is constrained by the fact that repetition of experiments with a similar batch of compost is impossible, since storage affects the organic material including the microbial communities. The objective of this study was to test the hypothesis that differential changes in microbial community structure and associated microbial activities after various storage methods (drying, freezing and cooling) lead to differential changes in the disease suppressive ability of compost material with respect to *Fusarium oxysporum* f. sp. *lini* induced by mixes of composts with peat substrate (20/80%, vol./vol.). A significant ($P<0.0001$) storage method × compost interaction was found with respect to suppression of Fusarium wilt of flax, indicating that the effect of storage type on disease suppression is compost-dependent. For seven composts storage had no (13 cases) or a significantly positive effect (eight cases) on disease suppression and for 1 compost there was a significant negative effect of storage on disease suppression. Significant changes in microbial activity and 16S-rDNA DGGE banding patterns of the composts were observed as a result of all tested ways of storage and these changes could be related to changes in disease suppression: relatively strong changes in microbial activity and bacterial composition due to storage had a relatively strong effect on disease suppression. The cool storage treatment (4 °C) resulted in the least deviation in disease suppression from the fresh compost, although the freezing treatment gave the most reliable results with the lowest standard deviation.

Keywords: Compost; Disease suppression; Microbial communities; DGGE; Microbial activity; Cool; Frozen; Dry; Storage; *Fusarium oxysporum*

1. Introduction

Composts have been shown to enhance disease suppression against soilborne plant pathogens in peat or soil mixes (reviewed by Hoitink and Boehm, 1999; Scheuerell et al., 2005; Termorshuizen et al., 2006). A factor constraining compost research is that repetition of experiments with the same batch of compost is difficult since during storage further decomposition of the organic material always occurs. This topic is also relevant for the predictability of disease suppressiveness of commercial composts that are stored in bags until they are used. Storage of compost implies phenomena like drying-rewetting, freezing-thawing, or cooling-returning to room temperature, depending on the type of storage. These have been studied for soil samples (e.g. Pesaro et al., 2004; Sharma et al. 2006; Koponen et al., 2006), but much less so for compost (Mondini et al., 2002; Yang et al., 2004).

Microbial characteristics of compost or soil likely change as a result of storage. Microbial biomass generally declines during drying, freezing and cooling and microbial activity declines sharply. Only during cooling microbial activity continues to some extent (Jenkinson and Powlson, 1976; Butler et al., 2001). After revival through rewetting, thawing or returning to room temperature, typically the microbial biomass is reduced for a prolonged period of time (Schimel and Clein, 1996; Stenberg et al., 1998; Pesaro et al., 2004). After drying-rewetting and freezing-thawing, dead cells are mineralized and non-biomass nutrient sources become available as a result of physical disruption of the substrate, causing a temporary strong increase in microbial activity (van Gestel et al., 1993; Scheu and Parkinson, 1994; Pesaro et al., 2004). For example, in

*Corresponding author. Tel.: +31 317 48 59 24; fax: +31 317 47 82 13.
E-mail address: Etienne.vanRijn73@gmail.com (E. van Rijn).

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experiments on soil, freezing-thawing led to a 47% increase of substrate-induced respiration (Pesaro et al., 2003) after which it returned to reference levels within 10 days, but microbial biomass, as estimated by direct cell counts and soil DNA content, was reduced by 24–33 and 15–23%, respectively, until at least 42 d after thawing. Typically the microbial communities show a significant shift in their composition due to storage as a result of changes in the substrate and because some species may be more sensitive to stress invoked during drying-rewetting (Scheu and Parkinson, 1994; Pesaro et al., 2004) or freezing-thawing (Yang et al., 2004; Sharma et al., 2006) than others.

Compost-induced suppression of Fusarium wilt of flax, caused by *Fusarium oxysporum* f. sp. *lini*, has been reported by several researchers for a wide range of different composts (Serra-Wittling et al., 1996; Cotxarrera et al., 2002; El-Masry et al. 2002; Borrero et al., 2004; Termorshuizen et al., 2006) and is, at least partly, competition-based (Serra-Witling et al., 1996; Borrero et al., 2004; Termorshuizen et al., 2006). As changes in the microbial community as a result of storage are likely to happen and the disease suppressive properties of compost/peat mixes are, at least in part, driven by its microbial activity and composition (Hoitink and Boehm, 1999), the question arises to what extent compost storage affects disease suppression. The goal of this research was to test the hypothesis that differential changes in microbial community structure (as determined by DGGE) and associated microbial activities (measured as basal respiration) after various storage methods (drying, freezing and cooling) lead to differential changes in the disease suppressive ability of compost material with respect to Fusarium wilt of flax.

2. Material and methods

2.1. Collection and storage of composts

Composts were collected from six different composting sites (Table 1), sieved over a 10-mm screen and brought to 50% water holding capacity (WHC) using tap water. These composts were tested for disease suppression 2 d after collection and for basal respiration after an incubation period of 11 d as described below. The remainder of the composts (3 l) was put in 6-l polyethylene bags. The bags were loosely closed to allow oxygen exchange. During 12 week, the following three storage treatments were carried out: (1) cooling: +4 °C storage, (2) freezing: −20 °C storage, and (3) drying: storage at 20 °C after drying the composts for 24 h at 60 °C. At the end of the storage incubation, frozen and cooled samples were brought back to room temperature and all plastic bags were checked for their moisture content and wetted to 50% WHC if needed.

2.2. Physical and chemical analyses

WHC was determined according to Alef and Nannipieri (1995). Measurements of pH and EC were carried out with a pH/mV meter (InoLab, Germany). Air-dried pure compost samples (2 g, sieved over a 2 mm sieve), fresh or stored, were added to 100 ml 0.01 M $CaCl_2$ solution and shaken for 2 h. The organic matter of the samples was assessed gravimetrically by dry combustion of the organic material in a furnace at 500–550 °C. Total N and C were measured with a Fisons Type EA 1108 Element Analyzer (Milan, Italy) according to the method of Dumas (Anonymous, 1997).

2.3. Microbial activity

Plastic bags with fresh compost or compost that was rewetted or returned to room temperature were placed for 11 d at 20 °C to restore microbial activity. Basal respiration of the composts was determined with an automated system in which a continuous air flow of 50 ml min^{-1} was led over 30.0 g f.w. of compost in glass tubes (length 24 cm, diam. 3.5 cm) incubated at 20 °C for 24 h. The CO_2-concentration in this air stream was measured by means of a computer-controlled switching device and an infrared CO_2-analyser (ADC 7000, Analytical Development Corporation, Hoddesdon, UK) which allowed hourly measurements. For calculation of the basal respiration the readings of the

Table 1
Origin of the composts, used in disease suppression experiments

Site	Compost sample	Disease suppression bioassay[a]	Compost age (wk)[b]	Composting method	Raw materials
1	a	1	2	Static indoors pile	Organic household waste (vegetable, fruit and garden waste)
	b		8		
2	c	1	10–12	Static outdoors pile	Leaves and wood trimmings
3	d	1	10–12	Static outdoors pile	Leaves and wood trimmings, manure, clay
4	e	2	2	Tunnel Indoors	Organic household waste (vegetable, fruit and garden waste)
	f		8		
5	g	2	10–12	Tunnel Indoors	Organic household waste (vegetable, fruit and garden waste)
6	h	2	10-12	Static outdoors pile	Leaves and wood trimmings

[a]Bioassays were carried out at two different times; 1 and 2 were started on 10-04-2005 and 9-06-2005, respectively.
[b]Compost age at time of sampling.