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Can inoculation with living soil standardize microbial communities in soilless potting substrates?

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ABSTRACT

Soilless plant cultivation substrates are commercially produced at large scales, but can show considerable variation in their performance in terms of sustaining plant growth and/or nutrition. This variation may be due to varying composition of microbial communities present in the substrates, mainly when composted organic materials are used as their components. Here we analyzed the portion of variability in composition of microbial (mainly the fungal) communities due to identity of substrate batches and compared it with variability due to the addition of a living soil (inoculation) or the presence of plant root system (i.e., the rhizosphere effect). The analysis was based on profiling total (DNA-based) and active (RNA-based) fungal and total (DNA-based) bacterial communities by using cultivation-independent molecular approaches. Contrary to expected effect of inoculation and rather limited variation across the substrate batches, identity of substrate batches in fact turned to explain the largest portion of biological variability, followed by the rhizosphere effect. The inoculation was completely ineffective as a factor affecting the indigenous microbial communities. These results indicate that the microbial communities in the soilless substrates are particularly resilient to plant- or inoculation-induced changes, but still highly variable between the individual production batches. Active fungal communities were dominated by yeasts recruiting either from Asco- or Basidiomycota. Due to phylogenetically and functionally similar but mutually exclusive dominants (*Galactomyces* and *Candida*) of the microbial communities in the different substrate batches without obvious correlation with their physico-chemical properties, we assume functional redundancy to play an important role in microbial community assembly within the substrates. Our results thus demonstrate as yet undescribed variation in microbial community composition with possible functional impact on plant performance in soilless substrates deserving further experimental attention.

1. Introduction

Many plants rely to a large extent on services provided by specific soil microbes such as rhizobia, mycorrhizal fungi and/or fluorescent pseudomonads for their nutrition and tolerance to pathogens and/or environmental stresses. Recognition of these beneficial plant-microbial interactions has since a long time stimulated research efforts to increase abundance of such specific microbes in various ecosystems for improved agricultural and/or horticultural production, mainly through inoculation (Püschel et al., 2014; Berruti et al., 2016). However, the desired effects have not always been observed following the introduction of the selected microbes into the soil environments (Koltai, 2010). Poor matching of microbial strains to their environment is probably the reason for the majority of these failures. Therefore, uncovering mechanistic basis of the microbial-environment matching and using this knowledge in further efforts to manipulate microbial communities in specific environments is the next research challenge.

Soilless (mainly peat- or compost-based) substrates, which are produced at large (industrial) scales and are widely used in gardening and horticulture, represent an important market commodity. Yet, their biological activity in terms of promoting plant growth and/or disease suppression can vary substantially, even across the batches of the same substrate type (StMartin and Brathwaite, 2012). Such variability is partly due to inherent variation in physico-chemical properties between batches of

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natural products such as bark, peat, pumice and composts, which are used as components of the soilless substrates. Another important portion of this variation is probably due to differences in composition of microbial communities or their metabolic activities. This "biological value", i.e., contribution of microbial activity to improved plant nutrition and/or pathogen protection has often been documented in experiments, where the microbes were experimentally suppressed or inactivated by sterilization, e.g., by heating (Trifonova et al., 2009). Together, with variation introduced by irregularities within the industrial production, this leads to large variation of the performance of the final product (i.e., marketed substrate), leading to potential marketing and consumer satisfaction issues. This is mainly true for substrates containing composts (Herrera et al., 2008), which are used to partially or fully replace traditionally used peat.

Because of the above-mentioned issues, the manufacturers of soilless potting substrates are actively seeking for measures to stabilize quality of their products on a long run and to improve and maintain the performance of the substrates (Sonneveld and Voogt 2009, chapter 11). In spite of some previous encouraging results (Pane et al., 2011), the standardization of microbial communities present in the substrates have not received the appropriate systematic attention yet, as compared to other features like the availability of macronutrients, maintaining optimal pH and water holding properties. At the same time, however, substantial variation in microbial enzyme activities important for plant performance caused by the variation in the substrate composition have been reported (Hernandez and Hobbie, 2010). The major impediment to the use of compost as a component of substrates appeared to be high variation in physico-chemical properties and disease suppression levels across and within compost types, sources, and batches (StMartin and Brathwaite, 2012), which may be connected with the above variation in microbial activity. The question appears whether the defined composition of the substrate alone (i.e., maintaining the component ratios) can guarantee the stability of the substrate microbial activities and/or composition in the product. Because of the above concerns, inoculation of commercial soilless substrates with diverse microbial communities appears particularly attractive, yet has remained difficult to study directly until the recent development of high-throughput sequencing technologies (Fracchia et al., 2006).

One of the major components of commercial substrates for ornamental plants such as *Pelargonium*, *Petunia* and *Begonia* is spruce bark, besides other components such as different kinds of peat. Bark is relatively uniform in terms of physico-chemical properties (Chong, 2005), and the microbial community of the bark is supposedly rather homogeneous across the batches. The first goal of this study was to check validity of the above assertion by comparing the microbial communities across different substrate batches. Further, we asked the question whether addition of a living rhizosphere soil (inoculation) to a soilless potting substrate substantially impacted the structure of microbial communities in such a substrate in general and the microbial composition of rhizosphere developing in such a substrate in particular. The rationale of inoculating the substrate with a rhizosphere of unrelated plant is based on the fact that various rhizosphere microorganisms have often been heralded as beneficial microbial inoculants even across different plant species (Hrynkiewicz and Baum, 2012; Püschel et al., 2014), although the plant species-specific promotion of certain microbes in their rhizospheres has repeatedly been described in the past (e.g., Marschner et al., 2004). To this end, microbial communities (particularly, the fungal community that are supposed to play a more important role in composted organic materials, e.g. Wei et al., 2012) were compared between three batches of soilless substrate as affected by soil inoculation and presence of a plant (*Pelargonium* sp.). Both the total (DNA-based) and active (RNA-based) fungal and DNA-based bacterial communities were analyzed in the differently treated substrate samples by 454-amplicon sequencing and influence of the different factors on the microbial community profiles was disentangled using multivariate statistics.

2. Materials and methods

2.1. Substrate

In our study, we used three batches of a commercial potting substrate for balcony plants produced by "Rašelina a. s." (Soběslav, Czech Republic). The substrate is composed of three different kinds of peat (see Table 1 for details), spruce bark compost and finely milled dolomite (exact mixing ratios of the components were always the same across the different batches, but cannot be

Table 1
Physico-chemical properties of the three tested substrate batches before their incubation in the pots, their individual components, and the living rhizosphere soil used as microbial inoculum. Each substrate was prepared by mixing the respective batches of Components 1 through 4, using the same mixing component ratios.

	Batch	Name	Organic C (%)	Total N (%)	Total P (mg/g)	C/N ratio	pH (H_2O)
Potting substrate	1	S 1	40.19	2.33	1.12	17.24	6.1
	2	S 2	37.12	2.36	0.67	15.75	5.4
	3	S 3	37.25	2.22	0.76	16.76	5.4
Component 1 (spruce bark compost)	1	C 1	41.82	0.86	0.43	48.78	6.4
	2	C 2	41.36	0.81	0.41	51.37	6.2
	3	C 3	41.04	0.65	0.36	62.82	5.9
Component 2 (fibric white peat)	1	WP 1	42.50	0.91	0.24	46.62	3.9
	2	WP 2	45.49	0.80	0.12	57.20	4.0
	3	WP 3	44.82	1.75	0.14	25.55	3.8
Component 3 (sapric black marsh peat A)	1	BPa 1	41.76	2.64	0.52	15.82	4.2
	2	BPa 2	35.29	2.35	0.57	15.03	4.6
	3	BPa 3	34.67	2.14	0.58	16.23	4.2
Component 4 (sapric black marsh peat B)	1	BPb 1	42.35	2.63	1.02	16.11	4.4
	2	BPb 2	40.37	1.96	0.65	20.60	4.5
	3	BPb 3	47.67	2.14	0.39	22.25	4.4
Inoculum (living soil)	–	I	1.93	0.21	0.50	9.36	7.5