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Research article

Arbuscular mycorrhizal fungal community in the topsoil of a subtropical landfill restored after 18 years

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ABSTRACT

Restoration of disturbed habitats (e.g., landfills and mine tailings) is important to recover ecosystem services. Arbuscular mycorrhizal (AM) fungal community is an important indicator of ecological performance of ecosystems. Rhizospheric soils were collected in two sites (A1 and A2) within the restored area of a landfill (18 yrs after restoration), and two sites (B and C, serving as control) in the adjacent natural area. Soil properties were analysed. AM fungal communities in soils were analysed by sequencing 18S small subunit rRNA gene. Results showed that genera *Glomus* (the most abundant, relative abundance: 10–24%), *Paraglomus* and *Rhizophagus* were commonly found at all sites. *Acaulospora* and *Redeckera* were found exclusively at natural sites, while *Scutellospora* only at the restored site. On average, AM fungal species richness was lower (87 operational taxonomy units, OTUs), while diversity was higher (Shannon index 3.2) in restored site, compared with control (107 OTUs, Shannon index 2.8). The structure of the AM fungal communities was influenced by soil nitrogen and cation exchange capacity. The restored sites possessed a more phylogenetically heterogeneous fungal community than that in natural sites. AM fungal community at restored sites clearly deviated from that at natural sites, indicating that current restoration practice is certainly inadequate. The trend of ecological succession could be significantly influenced by rehabilitation methods, such as adjustment of initial soil properties and selection of plant species. This study highlights the necessity of assessing AM fungal community during ecological restoration for sustainable ecosystem, in addition to plant and bacteria.

1. Introduction

Building a sustainable city should take the ecological performance of the city into account. The functioning and stability of terrestrial ecosystems are determined by species diversity and composition. Arbuscular mycorrhizal (AM) fungi, which form symbiosis with > 80% of plants (Smith and Read, 2008), play an essential role in terrestrial ecosystems (Rillig, 2004). Understanding the actual status of these fungi in man-made ecosystems can offer important basic information on how they can affect soil properties, such as carbon content (Cheng et al., 2012; Smith and Read, 2008; Treseder and Allen, 2000), hydraulic conductivity (Feeney et al., 2004), wet-aggregate stability (Rillig et al., 2002), and water retention characteristics (Augé, 2001). These parameters are tightly linked to plant performance of these ecosystems. The AM fungal community also serves as an indicator of ecological performance of the ecosystem (van der Heijden et al., 1998). AM fungal diversity is a major factor contributing to plant diversity, ecosystem variability and productivity (van der Heijden et al., 1998). This emphasizes the need to pay attention to AM fungi in our man-made terrestrial environment in order to maintain diverse ecosystems. However, the status of AM fungi in man-made ecosystems has been largely ignored.

Conventional maintenance of geotechnical structures such as landfills and filled slopes does not take microorganisms into account. However, the fact that soil properties change over time due to ecological process (Burbank et al., 2013; DeJong et al., 2015) should not be

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ignored. Plants, bacteria, fungi, and burrowing animals (such as ants and earthworms) can have considerable influence on soil properties, including the amount of organic matter, void ratio, soil aggregate stability, hydraulic conductivity, and soil water retention characteristics. These subsequently affect the stability of the soil structure (Burbank et al., 2013). In fact, biological factors are being considered as important factors in geotechnical works (Burbank et al., 2013).

AM fungi generate significant amounts of organic matter, namely glomalin, which provides cohesion between soil particles forming aggregates (Bedini et al., 2009). It is important to understand which AM fungal genus/species can be established in a filled slope, which usually possesses a high degree of soil compaction (safety concern) that leads to relatively poor plant performance. Therefore, the restored area within the South East New Territories (SENT) landfill in Hong Kong was selected for identifying AM fungal species in the man-made ecosystem. The topsoil of the landfill cover might naturally contain AM fungi and is readily associated with plants. The objectives of the present study were to 1) study the status of the AM fungal community within the restored landfill site, comparing to a natural area, and 2) explore the contributing factors on the structure of the fungal community, in order to facilitate future restoration management.

2. Materials and methods

2.1. Site description

The restoration programme for the final cover of SENT landfill acquired 16 plant species to grow on completely decomposed granite (CDG) soil mixed with compost. Both native and exotic species were included. Exotic species were included due to their faster growth and higher survival rates (Wong et al., 2015). After 18 years of restoration (1998–2015), the SENT landfill offers an invaluable area for studying the ecological performance of man-made ecosystems. Throughout the years, plant and animal communities in the restored area have been monitored (Chen et al., 2016; Wong et al., 2016). The biodiversity and richness of microbes (such as AM fungi and bacteria) in the topsoil were not considered. Until 2015, bacterial community compositions were investigated (Chen et al., 2017).

2.2. Soil sampling

Soil samples were collected at two sites (A1 and A2) within the restored area, and two sites (B and C, serving as control) in the adjacent natural area (Fig. 1). A1 and A2 are approximately 100 m apart from each other. B and C are approximately 70 and 150 m away from the eastern boundary of the landfill, respectively.

A line transect (25 m) was randomly placed on each site. Soil samples (~500 g each) at the depth between 5 and 10 cm (rhizosphere) were collected, with five replicates at each 5-m interval along the transect. Each soil sample was well-mixed and put in a polypropylene (PP) sealed bag inside an ice box. All samples were then transported to the laboratory within one hour. Subsamples (~50 g each) were immediately placed in the 50 mL conical centrifuge tubes and stored at −80 °C prior to DNA extraction. Other subsamples were used for the analyses of soil physico-chemical properties.

2.3. Soil physico-chemical properties analysis

Soil moisture content (MC) was determined according to the method described by Klute et al. (1994). Other soil samples were air-dried, sieved (0.2 mm) and stored at 4 °C prior to further analyses. Soil properties including pH, electrical conductivity (EC), cation exchange capacity (CEC), total organic carbon (TOC), organic matter (OM), total nitrogen (N), total phosphorus (P), extractable N and extractable P were determined using the methods described by Sparks et al. (1996).

2.4. AM fungal community analyses

2.4.1. DNA extraction and target fragment amplification

Total genomic DNA in soil was extracted using the MOBIO PowerSoil® DNA Isolation Kit (MOBIO, San Diego, USA). Extracted DNA was used as templates to amplify a region (length 300 base pair) within the 18S SSU rRNA gene of AM fungi using the following primers (Van Geel et al., 2014):

AMV4.5NF: 5′-AAGCTCGTAGTTGAATTTCG-3′
AMDGR: 5′-CCCAACTATCCCTATTAATCAT-3′

Barcodes for sample multiplexing during sequencing were added at the 5' terminus of the forward primer. Dual-index barcodes and sequencing adapters were attached to the amplicon using NEBNext® Multiplex Oligos for Illumina® (NEB, San Diego, CA, USA).

The target DNA region was amplified in a 50-μL reaction system containing 1 μL of each primer (10 mM), 1 μL of genomic DNA, 0.5 μL of polymerase, 4 μL of 10 × Taq buffer with KCl, 4 μL of deoxynucleotide triphosphates mixture (2.5 mM) and 38.5 μL of nuclease-free water. The thermal cycling programme of the PCR was an initial denaturing step at 94 °C for 5 min; 30 cycles of denaturing at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 45 s; for the 30th cycle, the final extension was held at 16 °C (Bio-Rad T100 Gradient Cycler, Bio-Rad, USA). The products were then purified using the OMEGA Gel Extraction Kit (Omega Bio-tek, Norcross, GA) and quantified using a Qubit®2.0 fluorometer (Life Technologies, Shanghai, China).

2.4.2. DNA sequencing and data analysis

PCR products were pooled and sequenced using PE300 (MiSeq, Illumina) according to the manufacturer's instruction (Illumina, San Diego, CA, U.S.). The raw reads containing one or more ambiguous bases and those contaminated by adapter were removed to ensure sequencing quality. The obtained sequencing results were analysed using QIIME v1.9.1 (Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2010). The sequences of the 18S SSU rRNA gene with 97% of similarity were classified into an operational taxonomic unit (OTU), using the open reference OTUs picking method. OTUs are pragmatic proxies for microbial "species" and are generally ecologically consistent across habitats (Schmidt et al., 2014). The SILVA 123 (Quast et al., 2013) was used as the reference database. The sequences failed to match the SILVA were further clustered using the de novo method (UCLUST) (Edgar, 2010). For each OTU, the most abundant sequence was selected as the representative. Taxonomy information was assigned to each representative OTU according to SILVA using QIIME.

2.5. Statistical analyses

Together with the phylogenetic tree, the OTU table (OTU counts per sample) was used to calculate the α and β diversity. Since the total reads of samples A2.2 and A2.5 were relatively low (2417 and 2896), these samples were excluded from subsequent analyses. For each sample, the relative abundance (%) of each OTU was calculated using the number of the sequences clustered into such an OTU, divided by the total number of all sequences clustered. Species richness was counted as the number of OTUs derived from each sample. Depending on the normality of the data, Kruskal–Wallis one-way ANOVA (non-parametric) or One-way ANOVA followed by Duncan's multiple range tests (parametric) were performed to separate the differences between groups at the 95% confidence level.

Non-metric multidimensional scaling (NMDS) analysis (based on Bray-Curtis distance matrix) and analysis of similarities (ANOSIM, 999 permutations, nonparametric) were used to investigate the dissimilarity of communities among sites using PRIMER v6 (Clarke and Gorley, 2006). Principal coordinates analysis (PCoA) was conducted using QIIME v1.9.1 (based on unweighted UniFrac distance matrix)