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Impact of urban land use on the bacterial phyllosphere of ivy (*Hedera sp.*)

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HIGHLIGHTS

- Richness and diversity of urban and non-urban ivy phyllosphere are similar.
- Bacterial composition of urban and non-urban ivy phyllosphere differ greatly.
- Traffic-derived PM is significantly related with abundances of many dominant taxa.

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ABSTRACT

The surface of the aerial parts of the plant, also termed the phyllosphere, is a selective habitat for microbes. The bacterial composition of the phyllosphere depends on host plant species, leaf characteristics, season, climate, and geographic location of the host plant. In this study, we investigated the effect of an urban environment on the bacterial composition of phyllosphere communities. We performed a passive biomonitoring experiment in which leaves were sampled from ivy (*Hedera sp.*), a common evergreen climber species, in urban and non-urban locations. Exposure to traffic-generated particulate matter was estimated using leaf biomagnetic analyses. The bacterial community composition was determined using 16S rRNA gene sequencing on the Illumina MiSeq. The phyllosphere microbial communities of ivy differed greatly between urban and non-urban locations, as we observed a shift in several of the dominant taxa: *Beijerinckia* and *Methylocystaceae* were most abundant in the non-urban phyllosphere, whereas *Hymenobacter* and *Sphingomonadaceae* were dominating the urban ivy phyllosphere. The richness, diversity and composition of the communities showed greater variability in the urban than in the non-urban locations, where traffic-generated PM was lower. Interestingly, the relative abundances of eight of the ten most dominant taxa correlated well with leaf magnetism, be it positive or negative. The results of this study indicate that an urban environment can greatly affect the local phyllosphere community composition. Although other urban-related factors cannot be ruled out, the relative abundance of most of the dominant taxa was significantly correlated with exposure to traffic-generated PM.

1. Introduction

The leaf surface habitat, also known as the phyllosphere, supports diverse bacterial communities shaped by both plant factors and environmental conditions (Vorholt, 2012). In turn, the epiphytic bacteria can affect the host plant by, for example, preventing colonization of certain plant pathogens and encouraging plant growth (Vorholt, 2012). Furthermore, these bacteria are capable of degrading or detoxifying atmospheric pollutants, such as polycyclic aromatic hydrocarbons (PAH) (Yutthammo et al., 2010), and are considered an important source of airborne bacterial particles (Gandolfi et al., 2013; Lindemann et al., 1982).

Various culture-independent studies indicate that the host plant is an important factor for the composition of a phyllosphere

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community. In their study of 56 different tree species, Redford et al. (2010) showed that different tree species harbour distinct phyllosphere communities, since community variability between tree species exceeded the variability within one tree species. This principle was confirmed for trees in temperate and tropical climates and for Mediterranean perennials as well (Kembel et al., 2014; Kim et al., 2012; Laforest-Lapointe et al., 2016; Lambais et al., 2006; Vokou et al., 2012). However, the microbial composition within plant species can also be influenced by geographic location of the host plant (Finkel et al., 2011, 2012; Knief et al., 2010; Qvit-Raz et al., 2012; Rastogi et al., 2012). This geographic effect may be caused by climatic differences (Finkel et al., 2011) or the limited dispersal of the colonizing taxa (Finkel et al., 2012; Qvit-Raz et al., 2012). Moreover, some studies indicate that there are important microbial community differences between urban and non-urban locations. For instance, Jumpponen and Jones (2010) have previously found significant differences in the culturable fraction of the fungal phyllosphere communities of urban and non-urban oak trees in the USA, but they did not include bacteria. Smaller studies, based on the culturing of phyllosphere bacteria, revealed important differences between one site with heavy traffic pollution and one less polluted site (Brighigna et al., 2000; Joshi et al., 2008; Khanna, 1986). However, these studies included a limited number of sample locations and were based on culture methods, which only allow a small part of the phyllosphere diversity to be studied (Whipps et al., 2008).

The aim of this study was to explore differences in the phyllosphere communities of a common and evergreen plant species between an urban and a non-urban environment, using a culture-independent method. To this end, we sampled the phyllosphere of *Hedera sp.* (ivy) plants at three different locations in the city of Antwerp (Belgium) and at three locations outside of the city. A culture-independent approach based on high-throughput *16S rRNA* amplicon sequencing was used to determine the bacterial community structure of the phyllosphere of this ubiquitous evergreen climber. Furthermore, we investigated the relation between the bacterial community composition and local atmospheric pollution for all sampled ivy plants, using leaf magnetism as a proxy for particulate matter (PM) originating from traffic.

2. Materials & methods

2.1. Sample collection

The study was conducted in the province of Antwerp, Belgium. Six sampling locations were selected within a distance of 22 km of each other. We sampled at three different locations in the city of Antwerp ('urban locations') and at three locations in more rural areas ('non-urban locations') surrounding the city. The non-urban sampling locations were selected in quiet, green and parklike residential areas, at the edge of the nature reserve *Kalmthoutse Heide* (locations 1 and 2) and in the ancient forest *Zevenbergenbos* (location 3). All urban sampling locations (locations 4 to 6) were located in densely built-up areas and next to busy roads with medium to high traffic intensity (daytime average between 1050 and 2100 vehicles/h; SGS, 2010). Moreover, they were all within a distance of 200–370 m from a very traffic-intensive motorway (daytime average about 8000 vehicles/h; SGS, 2010). The mean atmospheric concentrations of NO_x, PM_{10} and $PM_{2.5}$ modelled by ATMOSYS (www.atmosys.eu) for 2013 (the most recent available data) were consistently lower at the non-urban locations compared to the urban locations (Table 1). The soil texture of the sampling locations was similar: sandy loam soils were found at all locations except for location two, where the soil was sandy (http://www.geopunt.be/). All locations were sampled on the same day (17 February 2015), to minimize the effect of other factors such as weather and season. All locations experience a maritime temperate climate, with lowest and highest mean temperatures of 3 °C in January and 18 °C in July, respectively, and annual mean rainfall of 900 mm (Royal Meteorological Institute, www.kmi.be).

At each location, three *Hedera sp.* (*H. helix* or *H. hibernica*; common name: ivy) plants were selected from which healthy, mature, vegetative, undamaged leaves were sampled at 1.5–2 m height. Leaves were cut with scissors using gloves, which were sterilized on site with 70% ethanol. For microbiological analysis, at least 200 cm^2 of leaves of each plant were put in a sterile 50 mL falcon (VWR) and transported to the lab. A field blank consisted of a falcon without leaves. In parallel, three leaf samples per plant were collected of about 100 cm^2 each for magnetic analysis and stored in paper envelopes for transport to the lab.

2.2. DNA extraction and 16S rRNA V4 amplicon sequencing

Phyllosphere microbes were extracted from the leaves upon arrival in the lab, 1–5 h after the samples were taken, by adding 20 ml of TE buffer (10 mM Tris, 1 mM EDTA, pH 8) to each falcon tube. To suspend the phyllosphere bacteria in the TE buffer, the tubes were alternately vortexed for 15 s at maximum speed with the Vortex Genie® 2 (MoBio) and 15 s manually shaken, four times in total. The leaves were removed and the remaining TE buffer was centrifuged at 4000 g for 10 min and most of the supernatant was discarded. The pellet and remaining supernatant were centrifuged again in 2 mL tubes at 8000 g for 10 min. The remaining supernatant was removed and the pellet was resuspended in 400 μL of Bead Solution (PowerFecal DNA Isolation Kit, MoBio) and stored at −80 °C for optimal preservation of DNA, until further processing.

DNA extraction of the samples was done with the PowerFecal DNA Isolation Kit, according to the manufacturer's instructions. DNA was also extracted from a falcon without leaves, but treated like the other falcons, to identify potential contaminants of the sampling procedure. Additionally, a DNA extraction was carried out solely with kit reagents to identify kit contaminants. To attain a DNA-sequence-based identification of the bacteria in the samples, a short suitable sequence was targeted and its numbers were increased to allow for Illumina sequencing. Hence, a PCR amplification of the V4 region of the 16S rRNA gene was done using barcoded primers (IDT) as described by Kozich et al. (2013). Primers with different barcodes (short artificial DNA sequences) were used for different samples, in order to attribute sequences to their original samples after sequencing. The Phusion High-Fidelity DNA polymerase (Thermo Scientific) was used to limit the number of errors introduced during PCR, as these errors may lead to misidentification of the bacteria. A PCR blank was included to confirm the absence of non-specific amplification. Each DNA extract was amplified in duplicate with different barcodes to assess technical variation. The resulting amplicons were purified with Agencourt AMPure XP PCR purification system (Beckman Coulter) and quantified using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). Based on these DNA concentrations, samples were pooled at equimolar concentrations into one tube and diluted to 2 nM. The pooled amplicons were sequenced at the Centre for Medical Genetics (Edegem, Belgium) with Illumina MiSeq, using the 500-cycles MiSeq Reagent Kit v2 (Illumina). The sequences obtained in this study are available in the European Nucleotide Archive database under study accession number PRJEB14262.

2.3. Bioinformatic analysis

The UPARSE pipeline (Edgar, 2013) was used to assemble the paired reads, conduct quality filtering, cluster sequences into