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Fungal succession in relation to volatile organic compounds emissions from Scots pine and Norway spruce leaf litter-decomposing fungi

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HIGHLIGHTS

- The fungal community succession was investigated in a litter bag experiment.
- Eight fungi species were isolated from Scots pine and Norway spruce needle litter.
- As many as 75 C_2–C_{15} fungal volatile compounds were identified by GC-MS.
- Most components detected in emissions were very reactive substances.

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ABSTRACT

Leaf litter fungi are partly responsible for decomposition of dead material, nutrient mobilization and gas fluxes in forest ecosystems. It can be assumed that microbial destruction of dead plant materials is an important source of volatile organic compounds (VOCs) emitted into the atmosphere from terrestrial ecosystems. However, little information is available on both the composition of fungal VOCs and their producers whose community can be changed at different stages of litter decomposition. The fungal community succession was investigated in a litter bag experiment with Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) needle litter. The succession process can be divided into a several stages controlled mostly by changes in litter quality. At the very first stages of decomposition the needle litter was colonized by ascomycetes which can use readily available carbohydrates. At the later stages, the predominance of *Trichoderma* sp., the known producers of cellulolytic enzymes, was documented. To investigate the fungi-derived VOCs, eight fungi species were isolated. As a result of gas chromatographic analyses, as many as 75 C_2–C_{15} fungal volatile compounds were identified. Most components detected in emissions were very reactive substances: the principal groups of VOCs were formed by monoterpenes, carbonyl compounds and aliphatic alcohols. It was found that production of VOCs by fungi is species specific: only 10 metabolites were emitted into the gas phase by all eight species. The reported data confirm that the leave litter decomposition is important source of reactive organic compounds under the forest canopy.

1. Introduction

Litter decomposition is an important stage that connects many processes by which carbon and nutrients cycle between plants and soil. It is also one of the major pathways by which atmospheric carbon fixed during photosynthesis is returned to the atmosphere mainly in the form of CO_2 and, to a lesser extent, as CO and volatile organic compounds, VOCs (Hanson and Hoffman, 1994). Nevertheless, the latter play a very important role in many global processes, such as changing of the oxidative capacity of the atmosphere (i.e. increase of ozone, HO_x radicals and concentrations of other photooxidants) and the balance of greenhouse gases, with possible consequences for Earth's climate (Atkinson, 2000). It is well documented that living vegetation is the principal source of atmospheric VOCs, with the upper limit of global phytogenic VOC emissions being equal to 1300–1500 Tg yr^{-1} (Isidorov, 1990; Guenther et al., 1995; Guenther, 2002). However, little effort has been made to investigate and estimate the VOC emissions in the

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course of leaf litter destruction, which is surprising. The potential importance of this source results from its gigantic scale: for land ecosystems, the litter dry mass is estimated to be (6–8) $\times$ 10^{16} g (Zavarzin, 1984; Warneke et al., 1999). According to some laboratory investigations, VOCs accounted for up to 2.5% of the carbon flux from litter (Ramirez et al., 2010). Even if we take into consideration a bi-directional exchange of VOCs (source–sink phenomenon), and that the sink (absorption by soil and microbial community) is equal to 80% of VOCs released by leaf litter (Ramirez et al., 2010), the upper limit of estimation will give us a value of 320 Tg yr^{-1} which is comparable with the global terpene emission from living vegetation (Guenther et al., 1995). However, so far the set of the data available on VOCs fluxes between leaf litter, soil and the atmosphere is too small to estimate the actual scale of this phenomenon.

Nevertheless, there are some field observations that confirmed the importance of volatile organics emission from leaf litter (Janson, 1993; Hayward et al., 2001; Bäck et al., 2010; Aaltonen et al., 2011; Greenberg et al., 2012). The measurements of the diurnal vertical profiles of terpene hydrocarbons in coniferous forests in Sweden and Russia (Petersson, 1988; Janson, 1992; Isidorov et al., 1999) showed a maximum of their concentrations in the near-ground air. More recently, Copeland et al. (2014) have registered elevated mixing ratios of acetaldehyde, acetone and monoterpenes toward the bottom of a Douglas fir forest canopy, which confirms the contribution from large quantities of forest-floor leaf litter. Emitted by leaf litter, VOCs are mostly products of metabolic activity of microorganisms, which is controlled by different factors: changes in temperature, moisture, litter quality and biotic factors, first of all by the structure of litter-decomposing microbe communities.

Other important but relatively unstudied aspect of litter-derived VOCs is their mediation in interactions at the plant–microbe and bacteria–fungi interface. Lately there has been a growing bulk of literature on the ecological role of microbial (both bacterial and fungal) VOCs. (Effmert et al., 2012; Bennett et al., 2013; Bitas et al., 2013; Junker and Tholl, 2013; Piecchulla and Degenhardt, 2014; Hung et al., 2015).

The data collected in the cited literature, as well as in many other publications indicate an important role of litter-derived VOCs in different environmental phenomena. In spite of that little information is available on their “producers” and chemical composition of emitted VOCs. The aim of our study was to fill up these gaps to some extent.

The prerequisites for this investigation were the observations during recent laboratory and field studies of leaf litter chemistry and litter-derived VOC emissions. Firstly it was found that leaf litter emitted a wide range of volatile organics into a gas phase (Isidorov and Jdanova, 2002; Isidorov et al., 2003). Secondly taking as an example European larch, Scots pine and Norway spruce litter, it was shown that “dead” plant material contained considerable amounts of volatile components as well as non-volatile but easy degradable substances that can be VOC precursors formed as a result of enzymatic reaction (Isidorov et al., 2005, 2010).

The subjects of this investigation were the changes in fungal community at the first stages of Scots pine and Norway spruce litter decomposition under natural conditions typical for Central Europe and determination, with the aid of an HS-SPME/GC-MS technique, of the chemical composition of VOCs produced by selected fungi species isolated from litter.

2. Experimental

2.1. Site description, exposure of needle litter in the field and sampling

The study was carried out in the arboretum Kopna Góra (53° 14′ N and 23° 29′ E) belonging to a large complex of the Puszcza Knyszyńska Forests. The site description, sample preparation and processing were described in detail previously (Isidorov et al., 2010). In short, brown needles from the needle generation to be shed were collected from pine (*Pinus sylvestris*) trees growing at the site by shaking their limbs. The spruce needles were collected from the Norway spruce (*Picea abies*) plantation. Thoroughly mixed needles were incubated in 30 sieve-like litterbags with terylene net (200 $\times$ 200 $\times$ 20 mm; mesh size of 1.5 mm) which were fastened to the native litter/moss layer in the two measurement plots beneath pine and spruce trees. Each litterbag contained 15 g of litter. Sampling was carried out a couple of times per year, and on each occasion three samples from each plot were collected. The samples were (after mixing) divided into several sub-samples to determine: litter mass loss, VOC emissions rate, chemical composition of extractive compounds. The results of these experiments were described in Isidorov et al. (2010). After 30, 282, 490 and 690 days of incubation, one of the sub-samples was used for microbiological investigations which belongs to the current research.

2.2. Fungal isolates

Isolation of litter decomposing fungi was carried out in triplicate by a similar procedure. About 0.5 g of litter transported to the laboratory was elaborately mixed (10 min) with 150 g sterile silica sand. The samples of contaminated sand were transferred on Petri plates and poured over by the Martin-Johnson culture medium. For each sample, ten plates were used for isolation. Fungal identifications were made based on morphological macroscopic and microscopic characteristics (Domsch and Gams, 1972, Domsch et al., 1981; Raper and Fennell, 1965, Raper et al., 1968).

For purification and subsequent analysis of volatile compounds, hypnal tips of selected fungal species (*Trichoderma polysporum, T. koningi, Penicillium purpurogenum, Penicillium minioluteum, Absidia glauca, Mortierella isabeliana, Thielavia terricola* as well as none-spore forming fungi) were cut with a sterile knife and transferred into a sterile glass vessel (0.25 L in volume) with the same culture medium and cultivated at 28 °C in the dark for seven days.

2.3. Chemical analysis

Volatile compounds emitted into the gas phase from fungi were collected by head space solid-phase microextraction and analyzed by means of gas chromatography–mass spectrometry (HS-SPME/GC–MS). The vessel was sealed hermetically with a lid supplied with an inlet port, and the rubber septum of the port was picked by a needle protected the SPME fused silica fiber. The fiber was coated with a Carboxen/PDMS stationary phase (Supelco Inc., Bellefonte, PA, USA). It was exposed to a head space gas phase for 120 min at the temperature of 20 $\pm$ 1 °C. To improve the sorption, two Teflon tube scraps of 2 cm in length were introduced into the glass vessel with growing fungus species and the vessel was intensively shaken every 15 min, causing gas phase mixing by the Teflon scraps. The collected VOCs were desorbed by introducing the SPME fiber into the injector of a GC–MS apparatus with an MSD 5973 mass selective detector (Agilent Technologies, USA). Gas chromatograph was equipped with a HP-5 ms fused silica column (30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness). The helium flow rate through the column was 1 mL min^{-1}. The initial temperature was 35 °C (5 min); later, it