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Exothermic processes in industrial-scale piles of chipped pine-wood are linked to shifts in gamma-, alphaproteobacterial and fungal ascomycete communities

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ABSTRACT

Fast growing softwood species such as pine are preferred for biomass-based heat as well as electricity production and stored in large quantities. A newly established outdoor pile of freshly cut pine-wood chips was monitored to analyze exothermic processes. Inside the pile, a mean temperature increase of up to 44 °C was measured after six days of piling which was paralleled by a decrease of O_2-concentration and an increase of CO_2-concentration. Thereafter four additional CO_2 maxima were observed, while O_2-concentration remained at ambient concentration. The fungal community structure remained almost unchanged after four weeks, while the bacterial community structure was characterized by continuous shifts over time. The rapid heating in the early stage of storage corresponded to high relative abundance of microbial strains belonging to the genera *Pseudomonas*, *Luteibacter* and *Caulobacter*, ascomycetous genera *Sphaeropsis* and *Cadophora* and basidiomycetous order *Polyporales* and genus *Sistotremastrum*. The late stage was composed by a broader diversity of microorganisms, and heating processes inside the wood pile were attributed mainly to physicochemical processes. Taken together, these observations suggest that the early bacterial and fungal communities are key players in exothermic processes and were replaced by a broader diversity of highly adapted microorganisms.

1. Introduction

Substituting biomass for fossil fuels in the generation of energy is an important strategy in order to mitigate climate change and enhance security of supply. Biomass as energy source is already applied in manifold ways and its economic importance is expected to increase rapidly all over the world within the next few decades (DOE 2007). In particular, electricity generation from biomass is estimated to double in Europe between 2007 and 2020 to a total of 46 TWh per year. At the same time 34 TWh heat per year will be used from combined heat and power facilities, approx. three times the quantity in 2007 (Nitsch 2008). Among other biomass sources, scrap wood and forestry residues will be the most common energy source in the next decade (Nitsch et al. 2004). Fast-growing wood species such as pine, poplar and others are preferred for biomass-based heat and/or electricity production. Therefore, outdoor storage of wood in large-scale (thousands of tons of materials) becomes an increasingly important issue. This kind of storage is typically located outside, without any coverage, on solid surfaces such as concrete. This enables the mechanized movement of wood on demand.

Storage of whole stems is uncommon for industrial purposes as they are mainly directly processed by the harvester to end product formats. During biomass production, stems with small diameter and low-value woody biomass are harvested and cut to chips or chunks and wood is primarily stored as comminuted parts (Evans and Finkral 2009). Once fresh biomass is chopped and piled, various processes take place, commencing with respiration of plant cells and microbial activity, followed by other physical and chemical transformations (Jirjis 2005). These processes result in heat release, which due to low conductivity of wood can generate high temperatures (>200 °C) and self-ignition (Kubler 1987). Recently, Ferrero et al. (2009) modelled thermal scenarios for large-scale wood piles to predict such self-ignition processes. The model predicted that self-ignition processes are only to be expected when they are initiated by microbial activity (Ferrero et al. 2009).

The main carbon source for microbial aerobic processes in wood piles are heterotrophic degradation of wood polymers as well as autotrophic fixation of carbon dioxide. It has been shown that

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freshly fallen leaves or other plant parts harbour a microbial community broadly different from those occurring during the later stages of decomposition (Frankland 1998; De Boer et al. 2005). Degradation of wood polymers is carried out in aerobic conditions, mainly by a defined group of white, brown and soft rot fungi and also, to a minor extent, bacteria (De Boer et al. 2005). Process-relevant microorganisms inside wood-chip piles are expected to originate from both atmospheric deposition and the microbial community present in the source material. Scholz and colleagues (2005) revealed by cultivation-dependent methods that fungi from small-scale piles of wood chips shift from mesophilic to thermophilic communities of ascomycetes, and that they are highly correlated to pile temperature as well as chip size (Scholz et al. 2005). Whilst cultivation-dependent methods can be used to study microbial succession, recent advances in molecular approaches offer an advantage. Combined with fingerprinting techniques, PCR of the 16S rRNA gene was often applied to describe spatio-temporal dynamics in the bacterial community structure in laboratory experiments and natural ecosystems (Liu et al. 1997; Schwieger and Tebbe 1998; Noll et al. 2005; Noll and Wellinger 2008). This is the first report of a cultivation-independent approach to analyse bacterial and fungal communities inside wood-chip piles, linking phylogenetic community structure to chemical and physical parameters of the substrate.

The aim of this research was to investigate bacterial and fungal community structure and composition and their impact on temperature change inside the pile and on the chemical composition of pine-wood debris. Therefore, gas formation and temperature change was monitored and linked to microbial community structure and chemical composition of wood debris during a 302 days storage period.

2. Materials and methods

2.1. Site and sampling description

A newly established wood-debris pile of freshly felled and chipped pine material was built close to Schwedt (Germany) in July 2007 with a size of $20 \times 15 \times 6$ m. Particle size of debris averaged $10 \times 5 \times 4$ mm and contained also bark. The initial water content of debris was approx. 35% (wt/wt). During establishment of the debris pile, ten steel tubes were included at three levels to allow repeatable gas measurements inside the pile.

Temperature was continuously measured at ten positions inside the pile (Fig. 1; positions 1 to 10) and for reference; two thermocouples (K-Type) were also located outside the pile to measure ambient temperature. Every 30 min, temperature values were stored by data loggers (model TL 309, PCE Group, Meschede, Germany). Commencing at 160, 180 and 200 days after piling, a logger malfunction omitted temperature data for periods ranging from 10–20 days. To link the structure of the microbial community to decay processes in the pile, gas concentrations inside the pile were measured at four of the ten temperature measurement positions (Fig. 1; position 1, 6, 7 and 10) 6, 14, 21, 35, 49, 72, 101, 128, 146, 183, 195, 218, 253, 288 and 302 days after piling. *In situ* concentration of O_2 was measured with a gas analyzer (Servomex Gasanalysator 5200, Crowborough, East Sussex, UK) and a mobile FTIR-Spectrometer (Gasmet Model, Dx 4000N, Karlsruhe, Germany) was used to analyze the concentrations of CO_2, CO and CH_4. After gas measurements, the steel tubes were sealed.

At the same dates of gas measurements, as well as directly after piling, we collected approx. 20 g wood debris in five independent replicates 0.5 m and 2.0 m inside the pile, respectively. Samples were taken laterally at five different positions of the pile at a height of 1.8 m. The temperature at the sampling site and depth was recorded, and a 10 g portion of each sample was subsequently shock frozen by liquid nitrogen and transported to the laboratory in a cryogenic container. Additional 10 g portions were transported on ice in bags to determine the water content.

2.2. Characterization of wood debris

Since observable changes in the chemical composition of wood debris were expected to be slow, selected dates were chosen for chemical analysis of samples (directly after piling, 101, 128, 195 and 302 days after piling). To obtain homogenized wood debris, samples were finely ground with a planetary ball mill at $400 \times g$ without intervals for 5 min (Model PM100, Retsch, Haan, Germany). The total carbon-, nitrogen-, and sulphur contents were analyzed via elemental analysis (Model Vario Macro CNS, Elmentar Analysensysteme, Hanau, Germany) according to manufacturer's instructions. For pH measurements, 20 mL aliquots of distilled water were added to the samples and the solution was analysed with a pH meter (Model pH537, WTW, Weilheim, Germany). In addition, we analysed the chemical composition of samples using Fourier transform infrared (FTIR) measurements (Equinox 55, Bruker Optics, Ettlingen, Germany) coupled with an attenuated total reflexion (ATR) device (DuraSampl*IR*, Sens*IR* Europe, Warrington, UK) as described earlier (Naumann et al. 2007, Naumann 2009). FTIR absorbance peaks at relevant wavenumbers (cm^{-1}) were assigned to chemical bonds and compounds according to Faix (1991), Naumann (2000) and Pandey and Pitman (2003).

2.3. Microbial community fingerprinting analysis

Nucleic acids were extracted from all milled samples by applying the PowerSoil DNA isolation kit (Mo Bio Laboratoires, Carlsbad, CA, USA) according to the manufacturer's instructions. In preliminary experiments, several commercial DNA isolation kits were tested, as well as a non-commercial phenol-based DNA extraction method. The PowerSoil DNA kit recovered the highest amount of total nucleic acid and subsequent terminal-restriction fragment length polymorphism (T-RFLP) analyses as described in detail by Liu et al. (1997) detected a higher bacterial and fungal terminal-restriction fragment (T-RF) richness. The PowerSoil kit was therefore used in all subsequent analyses. Nucleic acid concentrations were determined photometrically with a microplate reader (Synergy HT, BioTek, Bad Friedrichshall, Germany). The 16S rRNA gene was amplified from 0.01 µl (approx. 0.5–2.5 ng environmental DNA) of environmental nucleic acid extract using the primers 27f and 907r (Lane 1991). The forward primer (27f) was 5′ labelled with 6-carboxyfluorescein (FAM). The fungal internal transcribed spacer (ITS) was amplified from the same nucleic acid extract concentrations using the primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The forward primer ITS1-F was 5′ labelled with FAM, while the reverse primer ITS4 was labelled with 4, 7, 2′, 4′, 5′, 7′-hexachloro-6-carboxyfluorescein (HEX). The PCR reaction (50 µl) contained 5 µl 10 × PCR buffer (5 Prime, Hamburg, Germany), 4 mM $MgCl_2$, 50 µM of each deoxynucleoside triphosphate (Invitrogen, Karlsruhe, Germany), 2.5 U *Taq* DNA polymerase (5 prime), 0.3 µM bacterial primers and 0.6 µM fungal primers (MWG Biotech, Ebersberg, Germany), respectively. PCR reactions were performed in a thermalcycler (Mastercycler, Eppendorf, Hamburg, Germany). The thermal profile of bacterial 16S rRNA gene amplification consisted of an initial denaturation step (2 min, 94 °C) followed by 30 cycles of denaturation (30 s, 94 °C), annealing (30 s, 48 °C), elongation (45 s, 72 °C) and a final elongation step at 72 °C was extended to 7 min. The thermal profile for fungal ITS amplification was performed as for the bacterial amplification, except for the number of cycles (35) and for the