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Naphthalene production by microorganisms associated with termites: Evidence from a microcosm experiment

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ABSTRACT

There have been several reports published which suggest that it is possible that the polycyclic aromatic hydrocarbons (PAHs) naphthalene (NAPH), phenanthrene (PHEN) and perylene (PERY) in tropical environments have a biological source. This source might be related to the activity of termites or their associated microorganisms. We aimed to provide direct evidence for the biological production of NAPH, PHEN and PERY by conducting microcosm experiments in the State of Tocantins, Brazil, in which termite nests (with or without termites) were placed in an enclosed environment in which we controlled all PAH fluxes and monitored changes of PAH stocks. The experiments were carried out with termites from a tropical floodplain forest environment at the Estação Canguçu (Ilha do Bananal) in the State of Tocantins, Brazil. We set up the following treatments: live nest of *Nasutitermes* cf. *minor* using PAH-poor wood as food (LNW), live nest of *Nasutitermes* cf. *minor* using PAH-poor corn as food (LNC), termite nest without live termites called dead nest (DNC) and dead nest with additional treatment by a combined fungicide/bactericide (FDN) in several replicates. In LNW, LNC, and DNC, there were mean increases of 43%, 21%, and 46% in NAPH stock after 20 d while the stocks of the 20 other PAHs studied did not change or even decreased. In contrast, FDN lost 20% of the NAPH stock after 20 d of the microcosm experiment because of dissipation and lack of microbial synthesis of new NAPH. In LNW and LNC, low-molecular weight PAHs (acenaphthylene to chrysene) were significantly lost at a mean percentage which was strongly correlated with the octanol–water partitioning coefficient (K_{OW}, $r = 0.78$). This was not the case in DNC and FDN. There were no indications that in the studied termite nests PHEN and PERY were produced. Our microcosm experiments suggest that NAPH can be produced by fungi and bacteria in termite mounds while all other low-molecular weight PAHs are degraded in microcosms with live termite nests. PAH degradation seems to be enhanced by the combined activity of termites and microorganisms.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a group of organic compounds containing at least two fused benzene rings in various arrangements (Blumer, 1976). PAHs are known to be hazardous (Menzie et al., 1992) and 16 of them are included in the US EPA class of priority pollutants (Keith and Telliard, 1979). The sources of PAHs in the environment can be anthropogenic or natural. Significant increases in PAH concentrations in dated sediment cores and archived soils since the onset of industrialization demonstrated that in the temperate industrialized world the main source of PAH contamination is a result of human activities, mainly combustion of fossil fuels (Jones et al., 1989; Fernandez et al., 2000; Elmquist et al., 2007).

Data on PAH patterns in the tropics are scarce (Connell et al., 1999; Wilcke 2000, 2007) even though such data are needed to understand the global PAH dynamics, because PAHs emitted into tropical environments may be globally distributed (Wania and Mackay, 1996). Recent studies in tropical environments indicate that there are differences in both the concentrations and the composition patterns of PAHs between tropical soils and soils of the

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industrialized part of the temperate zone. Tropical environments have lower PAH concentrations and are dominated by naphthalene (NAPH), phenanthrene (PHEN) and perylene (PERY) while industrialized temperate environments have higher PAH burdens and are dominated by high molecular weight PAHs (Wilcke 2000, 2007).

The high concentrations of NAPH, PHEN and PERY in tropical environments have been suggested to result from biological sources (Wilcke, 2000). An alternative explanation for the dominance of low-molecular weight PAHs (NAPH and PHEN) in tropical soils is that this may reflect higher source contributions from biomass burning in the tropics which emit more lower molecular weight PAHs, as well as preferential loss of lower molecular weight PAHs from temperate soils because of the higher age of their PAH loads (Dalya et al., 2007). An exact source assignment is still lacking. There are, however, increasing indications that biological production of NAPH is possible. These include reports of NAPH production by flowers of members of the plant genus *Magnolia* from Japan, China and North America (Azuma et al., 1996) and the plant family *Annonaceae* from the Amazonian rain forest (Jurgens et al., 2000) and by *Muscodor vitigenus*, a fungus from the Amazonian region of Peru (Daisy et al., 2002). Significant NAPH concentrations have also been detected in *Coptotermes formosanus* termite nests (Chen et al., 1998a,b), while NAPH, PHEN and PERY dominate in nests of other termite genera and plants sampled from Brazil (Wilcke et al., 2000, 2003, 2004). Dalya et al. (2007) reported high concentrations of PAH in air samples collected from the surroundings of termite nests. Perylene is known to be produced under anaerobic conditions by microorganisms in soils and sediments (Venkatesan, 1988; Guggenberger et al., 1996). These pieces of evidence led to the hypothesis that the partly high concentrations of NAPH, PHEN and PERY in tropical environmental media may be related to biological production by termites or microorganisms (i.e., fungi and bacteria) associated with termites (Wilcke, 2007).

Naphthalene is believed to play a role in the chemical defence against biological enemies (Wiltz et al., 1998; Wright et al., 2000). It may be produced by metabolic processes in termites or by associated microorganisms which inhabit, e.g., the termite guts (Varma et al., 1994). Direct evidence of the production of NAPH, PHEN or PERY by termites or associated microorganisms can be obtained with the help of microcosm experiments, in which termites are held in a closed environment under a PAH-free atmosphere. Microcosm experiments in which termite groups are fed with different food resources under controlled conditions have already been successfully used in ecological and ecotoxicological studies (Morgan and Knacker, 1994; Knacker and Römbke, 1997; Sheppard, 1997). Microcosm experiments with termites are furthermore a standard method to determine food consumption rates (Martius, 1997), food selection (Bustamante and Martius, 1998), and effects of pesticides (Su et al., 1987).

The aim of our study is to provide for the first time direct evidence of a potential production of NAPH, PHEN and PERY in termite nests. For this purpose, we ran microcosm experiments in Brazil in which we held termites under controlled environmental conditions. To separate the role in the potential PAH production of the termites themselves from that of microorganisms in the guts of the termites and microorganisms just inhabiting the termite mounds we also set up microcosms with dead nests which were either kept untreated in the microcosm and thus contained an intact microflora or treated with biocide to markedly reduce the activity of microorganisms.

2. Materials and methods

2.1. Study site

Termite nests used for the microcosm experiments were collected from the periodically flooded forest at the Estação Canguçu (Ilha do Bananal), Federal State of Tocantins, Brazil. The termites belong to the species *Nasutitermes* cf. *minor* which are potential wood feeders (Martius, 1994).

Soil material (loamy clay, 5 g organic C kg^{-1}, 12% water content, subsoil usually used for pottery) and corn (*Zea mays* L.) used for the microcosm experiment were collected from Taquaruçu, a small town near the city of Palmas, Federal State of Tocantins, Brazil. The soft wood samples, *Tilia platyphylla* C. A. Mey. were collected in Germany, because there are some indications that tropical trees may contain NAPH and PHEN (Wilcke et al. 2000; Krauss et al. 2005). Microcosm experiments were conducted in the laboratories of the Federal University of Tocantins in Palmas, Federal State of Tocantins, Brazil to keep transport distances as short as possible and to avoid strong changes of climatic conditions.

2.2. Microcosm experiments

The basic design of the microcosms consisted of a rectangular stainless-steel box with a height of 35 cm, a width of 35 cm, and a depth of 25 cm and a front plate made of transparent glass with an inlet and an outlet tube. The nests were taken from the forest and placed into the stainless-steel boxes on about 2000 g of loamy clay material (to better control the humidity in the microcosms). Before being placed in the microcosms, the nests were temporarily left in the forest until the outer wall, which was slightly damaged during the collection procedure was repaired by the termites. The food source of termites in rain forest is wood or soil or a combination of it depending on the species (Martius, 1994). We supplied wood (*T. platyphylla*) or corn (*Z. mays*) to serve as food for the termites. After termite nests, soil, wood/or corn had been placed in the rectangular boxes, a transparent glass plate was used to seal the front of the boxes and glued air-tight with silicon except for the two channels left as inlet and outlet for air. The inlet was connected via a tube to a pump for air supply to the microcosm and the outlet was connected to an outflow tube serving as an exhaust for outflowing air. In front of the inlet tube, XAD-2 cartridges were placed to clean the inbound air of PAHs, while at the outlet similar XAD-2 cartridges were placed to trap air-borne PAHs released in the microcosm. The XAD-2 cartridges were selected based on preliminary experiments in which activated charcoal was shown to be unsuitable. Gaseous PAHs are commonly sampled with XAD-2 resins (Liu et al., 2001; Krauss et al., 2005).

We set up six treatments as shown in Table 1, two of which were without termites and thus served as controls. For the two treatments with live termites, the nests (104–927 g dry mass) were placed on a soil layer (1843–2690 g dry mass) and supplied with corn (149–165 g dry mass) (LNC) or *T. platyphylla* wood (172–206 g dry mass) (LNW). For the two controls without live termites (DNC, FDN) the nests were separated into various parts and the termites manually removed completely. After removal of termites, the parts were combined again to minimize the surface area. One set of the dead nest was additionally treated with a combined bactericide and fungicide sold with the market name "Lysoform spray" (Milana

Table 1
Overview of the microcosm treatments. "Live nest" denotes a termite mound inhabited by termites, "Dead nest" is a termite mound not inhabited by termites.

Food	Treatment	Replicates	Mean dry mass (without cage) [g]
Corn	Live nest with corn (LNC)	3	2676
Tilia wood	Live nest with wood (LNW)	3	2966
Corn	Dead nest with corn (DNC)	2	3028
Corn	Fungicide/bactericide-treated dead nest (FDN)	2	2944
Corn	Blank corn-without nest (BC)	1	1949
Tilia wood	Blank wood-without nest (BW)	1	2339