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Root-associated branched tetraether source microorganisms may reduce estimated paleotemperatures in subsoil

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ABSTRACT

Branched glycerol dialkyl glycerol tetraethers (GDGTs) are complex lipids of high molecular weight, recently discovered in soils and suggested to be produced by still unknown bacteria. The relative distribution of these compounds was shown to depend on environmental parameters, mainly temperature and pH. Over the last years, an increasing number of studies have focused on the application of branched GDGTs as paleoclimate proxies, but only a few were performed in terrestrial archives. In this study, branched GDGTs were analyzed in calcified and non-calcified living and dead roots, in surrounding soil and sediment and in reference material distant from the roots. Samples were mainly collected from subsoil of two forest sites near Sopron (Hungary), where soils developed on fluvial sand and loess deposits, respectively. Branched GDGTs were more abundant in root samples and/or surrounding rhizosphere compared to reference material, suggesting that branched GDGT source microorganisms are closely associated with the root surface. In subsoil, the GDGT-based temperature estimates from former roots and surrounding sediments were mainly lower than those from reference material in both loess and sand profiles. This is likely due to the post-sedimentary incorporation of branched GDGTs deriving from microorganisms that fed on root organic matter in terrestrial sediments. In contrast, branched GDGT-derived temperatures do not seem to be influenced by the presence of roots in topsoil, which may be related to the much higher density of recent roots in topsoil than in subsoil. This argues for a more homogeneous distribution of root-associated microorganisms especially in densely rooted topsoils. In addition, we show that sample pre-treatment may have an effect on the abundance and distribution of branched GDGTs. Indeed, washing root samples with ultrapure water might lead to a decrease in GDGT abundance and an increase in temperature estimates, likely due to the removal of particles adhering to the root surface. Decarbonatization of root and surrounding sediment had only a limited effect on GDGT-derived parameters. Taken together, these results suggest that paleoenvironmental data obtained from branched GDGTs in terrestrial archives might depend on the way the samples were collected and prepared and should be interpreted with caution, especially in loess-paleosol sequences where the frequency of calcified roots can be locally very high.

1. Introduction

Glycerol dialkyl glycerol tetraethers (GDGTs) are membrane lipids of Archaea and some bacteria (Schouten et al., 2013 and references therein). Archaeal membranes are formed predominantly by isoprenoid GDGTs with acyclic or ring-containing biphytanyl chains. More recently, another type of GDGTs with branched instead of isoprenoid chains was discovered in peat deposits and also in soils (Fig. 1; Sinninghe Damsté et al., 2000). They were suggested to be produced by still unknown bacteria in soils, even though some of them might belong to the phylum Acidobacteria (Weijers et al., 2009; Sinninghe Damsté et al., 2011). Branched GDGTs are ubiquitous compounds in soils and peats (Weijers et al., 2007; Huguet et al., 2013) as well as in lacustrine (Pearson et al., 2011) and marine (Kim et al., 2007) environments. They are the object of a growing interest, especially because their relative distribution was shown to depend on environmental parameters, mainly mean annual air temperature (MAAT) and soil pH (Weijers et al., 2007). Two indices were developed based on the relative abundance of the different branched GDGTs: the MBT (Methylation index of Branched Tetraethers), which correlates with MAAT and to a lesser extent with soil pH, and the CBT (Cyclisation ratio of Branched Tetraethers), which depends on soil pH (Weijers et al., 2007). The MBT and CBT indices can therefore be used to reconstruct past air temperatures and has notably been applied in continental (Peterse et al.,

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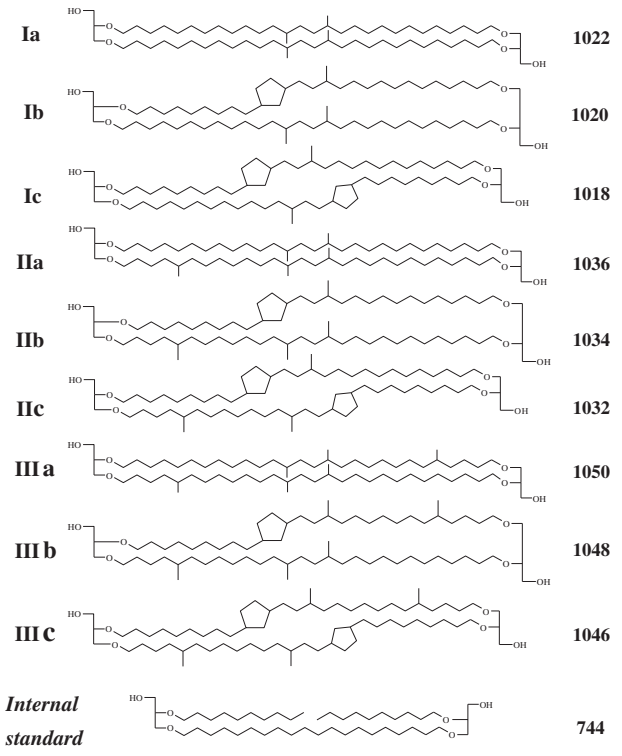

Fig. 1. Structures of branched glycerol dialkyl glycerol tetraether (GDGT) membrane lipids and internal standard (IS).

2011), coastal (Rueda et al., 2009) and lacustrine (Tierney et al., 2010) environments. Peterse et al. (2012) recently extended the original soil dataset used by Weijers et al. (2007) to 278 soils distributed worldwide. They proposed a new calibration to reconstruct soil pH and MAAT, based on the CBT and a simplified form of the MBT defined as MBT′.

Even though over the last years an increasing number of studies have focused on the potential use of branched GDGTs as paleoclimate proxies, the ecological niche of their source organisms remains still unknown. Branched GDGT-producing bacteria were suggested to be heterotrophic microorganisms, based (i) on similar stable carbon isotopic values for branched GDGT-derived alkanes and bulk organic carbon in soil (Oppermann et al., 2010; Weijers et al., 2010), (ii) more recently on higher branched GDGT abundance in calcified roots (rhizoliths) than in reference material distant from the roots in terrestrial sediments (Huguet et al., 2012), and (iii) also on their association with recent roots (Ayari et al., 2013). In the latter two studies, both, branched GDGT distribution and abundance were shown to differ between, on the one hand, roots and, on the other hand, surrounding terrestrial sediment and soil. However, it remains questionable whether this effect is also observed throughout the soil profile and within other environments than the two previously investigated sites (Huguet et al., 2012; Ayari et al., 2013). Thus, an improved knowledge of the habitat and lifestyle of branched GDGT-producing bacteria is essential to interpret the environmental data derived from these lipids.

The aim of this study was (i) to obtain more information on branched GDGT source bacteria via the analysis of branched GDGTs in the vicinity of root systems of different ages and origins and (ii) to investigate the influence of root-associated branched GDGT source microorganisms on the estimated paleotemperatures deriving from these lipids. Calcified and non-calcified living and dead roots were selected for this study. The abundance and distribution of branched GDGTs as well as temperature estimates derived from these compounds were determined in root samples and surrounding soil/sediment collected from forest soils of two sites, where soils developed on fluvial sand or loess deposits near Sopron (Hungary). In addition, the effect of sample pretreatment (washing of the samples with ultrapure water and decarbonatization) on GDGT-derived parameters was also examined.

2. Material and methods

2.1. Study sites

Sopron (NW Hungary) is located at the outermost margin of the Eastern Alps in a basin filled with sediments (consisting of limestones, sandstones, conglomerates, fluvial sands and loess) of Miocene age and younger. The mean annual air temperature (MAAT) is 10 °C, mean annual precipitation (MAP) is 710 mm, with a maximum of both in June and July. Two profiles with different geological backgrounds were chosen for investigation in the current study near the city of Sopron.

Profile A (N 47° 44.471′E 16° 33.678′, 278 m a.s.l.) is located in the Dudlesz Forest N of Sopron with *Quercus petraea* as the dominant vegetation. It comprises a 1.25 m thick Luvisol (IUSS Working group WRB, 2006) developed on grayish fluvial sand (Fig. 2A). A conglomerate with carbonatic cementation marks the boundary between the topsoil and the soil parent material. The topsoil was rooted mainly to a depth of 70 cm, whereas the soil parent material contained several horizontal to sloped (angle accounted up to 30° from horizontal) calcified root mats of several cm thickness (Fig. 2C). These were not connected to the recent trees and likely originate from former vegetation.

Profile B (N 47° 40.250′E 16° 33.871′, 285 m a.s.l.) is located in a small, abandoned brickyard at the WSW margin of Sopron within a forest dominated by *Fagus sylvatica*. The clayey, pale yellow loess is covered by a 1.3 m thick Luvisol (Fig. 2D). Roots from the recent vegetation penetrated not only the soil, but were also growing in fissures in the subsoil and parent material down to a depth of at least 2.5 m, showing a more or less pronounced carbonatic encrustation (Fig. 2E, F).

2.2. Sampling and sample preparation

A recent pit was sampled at site A and recent man-made outcrops were sampled at site B. Profiles were cleaned prior to sampling by removing at least 20 cm from the front wall. To compare recent and ancient, as well as calcified and non-calcified root systems, we collected the following samples (summarized in Table 1):

At profile A, two non-calcified dead oak root samples (Ro1, Ro2), one of them penetrated by living fine roots of the understory vegetation (FRo2; Fig. 2B), were taken together with surrounding rhizosphere soil up to a distance of 3 cm (RoS2a) at a depth between 0.2 and 0.3 m below present surface. Additionally, rhizosphere material at larger distance was collected at 0.35–0.5 m depth (RoS2b). No real reference soil without root remains could be collected for these samples due to intense rooting of the topsoil by the understory vegetation. Further, three calcified root samples (i.e. rhizoliths) were collected from the subsoil at 1.5–1.8 m depth (Rh1, Rh2, Rh3). Rh1 was sampled with surrounding sand up to a distance of 3 cm, and additionally sand at 3–6 cm distance was collected (RhSa1a, RhSa1b). Sand free of visible root remains was collected 50–70 cm distant from the rhizoliths as a reference in duplicate (Sa1, Sa2).

At profile B, three sets of initially calcified roots (Ro3, Ro4, Ro5), including surrounding loess (RoL3, RoL4, RoL5) up to a distance of 3 cm, were collected at 1.8–2.5 m depth. One reference sample (L) without visible root remains was collected 50–70 cm distant from the calcified roots.

All samples were dried at 40 °C prior to further treatment or analysis. Afterwards, rhizosphere material was separated from root or rhizolith samples, and all soil and sediment samples were homogenized by crushing in a ball mill (Retsch, Germany) after carefully removing root or rhizolith fragments and small stones with tweezers. Fine roots were separated from the larger dead root by tweezers as far as possible to obtain samples Ro2 (large dead root) and FRo2 (recent fine roots