**Chemical degradation of thermally altered silicified organic matter during acid
maceration: a case study from the Lower Devonian Rhynie chert**

Frédéric Delarue ^{a*}, Thanh Thuy Nguyen Tu ^a, Rémi Duhamel ^b, Céline Paris ^c, François
Baudin ^d

^a *Sorbonne Université, CNRS, EPHE, PSL, UMR 7619 METIS, 4 place Jussieu, F-75005
Paris, France*

^b *Muséum National d'Histoire Naturelle, Sorbonne Université, UMR 7590, IRD, IMPMC, F-
75005 Paris, France*

^c *Sorbonne Université, CNRS, UMR 8233 MONARIS, 75005, 4 place Jussieu, F-75005 Paris,
Paris, France*

^d *Sorbonne Université, CNRS, UMR 7193 IStEP, 4 place Jussieu, F-75005 Paris, France*

*Correspondence to: frederic.delarue@sorbonne-universite.fr

ABSTRACT

The effect of standard acid maceration on organic matter (OM) from ancient silicified sediments remains undocumented. Early silicification favours preservation of organic moieties against thermal alteration over time. In this study, we investigated the effects of acid maceration on the structure of OM isolated from the Lower Devonian Rhynie chert. The structure of OM was investigated by combining Rock-Eval pyrolysis and Raman spectroscopy. Besides a loss of thermolabile organic matter owing to solvent extraction, Rock-Eval pyrolysis showed that standard acid maceration also causes a loss of C-H emissions at high pyrolysis temperature ($> 500\text{ }^{\circ}\text{C}$). The standard acid maceration procedure was also associated with the disappearance of the D4 and D5 Raman spectrum shoulders assigned to C–H bonds in aliphatics and bitumens, respectively, entrapped in the macromolecular network. Taken together, Rock-Eval pyrolysis and Raman spectroscopy indicate that standard acid maceration can lead to the chemical degradation of syngenetic hydro-carbonaceous moieties of OM isolated from ancient silicified and thermally altered sediments. In sediments having experienced early silicification, which hampers bitumen migration and favours pyrobitumen formation, we suggest that novel in situ molecular analytical techniques are required to provide a thorough examination of the syngenetic molecular content independent of the soluble/insoluble operational definition.

1. Introduction

The chemical composition of ancient organic matter (OM) attests to turning points in the evolution of life during the history of the Earth (Summons et al., 1988; Edwards et al., 1997; Love et al., 2008; Duda et al., 2016; Nguyen et al., 2019; Love et al., 2020). However, investigating the structural and chemical compositions of ancient OM is still a challenging issue as thermal alteration during geological times results in losses of pristine molecular and

elemental content. Since ancient sediments and metasediments are generally depleted in OM, isolation procedures have typically been employed before applying analytical techniques to investigate the chemical composition of ancient OM, notably at the molecular scale. Historically, in the field of organic geochemistry, isolation of organic-insoluble and mineral-free OM is performed through a standard acid maceration procedure involving maceration followed by successive organic solvent extractions (e.g., dichloromethane [DCM] and methanol [MeOH]) and demineralisation with acid (e.g., hydrochloric [HCl] and hydrofluoric [HF]). This physical and chemical maceration procedure (HMM) yields kerogen, which is defined as insoluble macromolecular OM (Durand, 1980; Vandenbroucke and Largeau, 2007). Several investigations have suggested that acid maceration does not significantly modify the structure of kerogens from ancient rocks (Larsen et al., 1989; Vandenbroucke and Largeau, 2007; Aboulkas and El Harfi, 2009). However, there is contrasting evidence that acid maceration of coals can lead to either a rise in carbon structural order (Zhang et al., 2016) or a decrease in hydrocarbon content (Tekely et al., 1987). Moreover, Kebukawa et al. (2019) demonstrated the contrasting effects of acid maceration by comparing the chemical structure of bulk chondrites to their corresponding insoluble OM fractions. Insoluble OM from type 3 chondrite was depleted in aliphatic moieties in comparison to the bulk type 3 chondrite starting material. However, the effect of acid maceration varied among the different chondrite groups studied (Kebukawa et al., 2019). Thus, according to current literature, it therefore seems that the chemical stability of thermally altered OM to standard acid maceration cannot simply be assumed.

Among ancient sedimentary rocks, silicified sediments are of interest as they are essential geological archives of biological evolution throughout Earth's history. However, there is often very little OM remaining in these sediments and acid maceration procedures are required to concentrate it before studying its molecular content. Occurring prior to cell lysis

and early degradation of organic matter, rapid silicification leads to a closed chemical system by reducing sediment porosity (Boyce et al., 2002; Ledevin et al., 2014), which favours preservation of organic remnants against thermal alteration (Alleon et al., 2016). Using geochemical micrometre-scale analysis tools, it was demonstrated that some silicified Precambrian organic-walled microfossils are composed of significant amounts of carbonyl, phenolic, carboxylic, hydroxyl, and amide functional groups, despite being subjected to peak temperatures of up to 300 °C (Alleon et al., 2016). However, standard acid maceration procedures applied to ancient silicified sediments have the potential to degrade syngenetic OM through solvent extraction and acid hydrolysis of bitumens and chemically labile organic moieties, respectively. Such degradation, if it occurs, implies that a substantial amount of the initial molecular content depicting the evolution of life, past environmental conditions, and/or thermal maturation may be lost in the process. Further investigations on the effect of standard acid maceration on the chemical structure of ancient thermally altered silicified OM are therefore required.

Our aim in this study was to investigate the effect of the standard acid maceration procedure on the chemical structure of ancient silicified OM. We selected the iconic Lower Devonian Rhynie chert, the earliest preserved terrestrial ecosystem owing to a rapid and complete silicification through siliceous hot-spring deposits (Trewin, 2003; Preston and Genge, 2010). Despite the exceptional preservation of fossil plants, fungi, insects, and other organisms, the overall organic carbon content of Rhynie chert is very low (Summons et al., 1996). It therefore typically requires acid maceration to access the molecular signature(s). The Rhynie chert therefore constitutes a case study to evaluate the impact of standard acid maceration on the chemical structure of ancient thermally altered silicified OM. To this end, OM was isolated according to two acid maceration procedures: (1) a high-manipulation maceration (HMM), which followed the standard treatments generally applied to geological

samples, and (2) a low-manipulation maceration (LMM), which minimized chemical degradation by avoiding organic solvents and HCl. Rock-Eval pyrolysis and Raman spectroscopy were used to assess the chemical structure of the OM isolated by each procedure. Comparison of the so-obtained chemical structures allowed us to estimate the minimum amount of chemical degradation imparted by HMM maceration.

2. Material and methods

2.1. The Rhynie chert

Situated in Aberdeenshire (Scotland), the Rhynie chert is a Konservat-Lagerstätte. It is hosted by the Dryden Flags Formation, which is characterised by a succession of Lower Devonian sedimentary and volcanic rocks (Rice et al., 2002). Pragian–earliest Emsian in age (407.1 ± 2.2 Ma; Mark et al., 2011), the Rhynie chert is composed primarily of microcrystalline silica and was deposited as siliceous sinter from subaerial hot springs systems (Rice et al., 1995).

2.2. High- and low-manipulation maceration procedures

HMM and LMM procedures were performed on similar portions of the same rock sample. HMM was performed on ~30 g of crushed rock. Solvent extraction was first performed on rock powder using a mixture of DCM and MeOH (2:1; v:v). Carbonates were then removed at room temperature using HCl (37%; reagent grade) to minimise the formation of fluorides during subsequent HF/HCl maceration. Samples were then centrifuged and washed with ultrapure water until reaching neutrality. Concentration of OM was achieved through acid maceration at room temperature in a mixture of HF (40%, reagent grade) and HCl (2:1, v/v; reagent grade). Samples were centrifuged and washed with ultrapure water to reach neutrality. Finally, HCl (37%; reagent grade) at 60 °C (24 h) was used to degrade

neoformed fluorides. After an additional step of solvent extraction using a mixture of DCM and MeOH (2:1, v/v), the isolated OM was again centrifuged/washed with ultrapure water until reaching neutrality. Samples were then air-dried at 60 °C after final rinsing in acetone. Hereafter, OM isolated by the HMM procedure is referred to as OHMM.

LMM was performed by first fragmenting ~30 g of rock samples into ~3 g rock chips. Rock chips were cleaned using ultrapure water and a mixture of DCM and MeOH (2:1, v/v) and were then placed directly in a Teflon vessel filled with HF (40%, reagent grade) at room temperature. After 48 h, successive centrifugation and rinsing steps using ultrapure water were performed until reaching neutrality. Samples were then air-dried at 60 °C. Hereafter, OM isolated by the LMM procedure is referred to as OLMM.

2.3. Rock-Eval pyrolysis

OHMM and OLMM were analysed using Rock-Eval 6 (Vinci Technologies) following the standard pyrolysis protocol described in Behar et al. (2001). Performed in a N₂ atmosphere, Rock-Eval pyrolysis comprises two steps: an isothermal phase held for 3 minutes followed by a rise in pyrolysis temperature from 300 to 650 °C at a rate of 25 °C/min. After pyrolysis, the residual material was then heated from 300 °C to 850 °C under purified air in an oxidation oven in order to calculate total organic carbon (TOC) value (see Behar et al., 2001 for further details about calculation procedure). Released hydrocarbons (HC) were continuously quantified by a flame ionisation detector (S1 and S2, for the first and second pyrolysis steps, respectively, in mg HC/g of sample) while released CO and CO₂ were continuously and simultaneously monitored by infrared detectors during both pyrolysis (S3CO and S3CO₂) and combustion (S4CO and S4CO₂). Quantification of the amount of effluents led to the determination of the TOC (wt%), of the Hydrogen Index (HI, defined as $S2 \times 100 / TOC$, in mg HC/g of TOC) and of the Oxygen Index (OI; defined as $S3 \times 100 / TOC$, in

mg CO₂/g of TOC). The pyrolysis temperature associated with the maximum release of hydrocarbons, called “TpK_{S2}”, was also determined.

2.4. Raman spectroscopy

Raman spectroscopy analysis was performed using a Renishaw inVia micro-spectrometer equipped with a 532 nm argon laser. The spectrometer was first calibrated using a silicon standard before each session. For each sample analysis, the laser was focused using a Leica microscope with a ×50 objective and the spectra were recorded in the 1000–1900 cm⁻¹ first order spectral window including the defect (D) and graphite (G) peaks (Fig. 1). The laser power at the sample surface was kept below 1 mW to prevent thermal alteration of isolated OM (Everall et al., 1991). Spectra acquisition was achieved after two iterations using a time exposure of 40 s. After linear correction of the baseline between 1000 and 1900 cm⁻¹ and spectra normalization, we identified Raman peaks according to the nomenclature defined in Romero-Sarmiento et al. (2014).

The heights of the sub-bands D1 (~1365 cm⁻¹), D4 (~1285 cm⁻¹), D5 (~1445 cm⁻¹) and G+D2 (~1600 cm⁻¹; Fig. 1) were then determined to compute the I_{D1}/I_{G+D2}, I_{D4}/I_{G+D2} and I_{D5}/I_{G+D2}, ratios. Slopes α_{D4} (determined between 1265 and 1300 cm⁻¹ Raman shift) and α_{D5} (determined between 1415 and 1445 cm⁻¹ Raman shift) were determined to geometrically evaluate the expression of D4 and D5 shoulders on the D band.

3. Results and discussion

The TOC of OHMM and OLMM samples was measured to determine the efficiency of rock mineralization through HMM and LMM, respectively. The TOC in all OHMM and OLMM samples was low (25.4% and 27.5% respectively; Table 1) suggesting that the preservation and/or neoformation of substantial mineral content had occurred during both

procedures. The OI was approximately 15 mg and 59 mg CO₂/g TOC in OHMM and OLMM, respectively (Table 1). The higher OI in OLMM is partially explained by the higher level of CO₂ emissions during the first isothermal step, showing that they are mostly related to desorbable OM (Fig. 2). Such a result is in line with previous investigations on coals suggesting that acid maceration can lead to a rise in oxygen content resulting possibly from the neoformation of carboxylic groups replacing carboxylate through ion exchange (Larsen et al., 1989). Higher levels of CO₂ emissions were also recorded for OLMM during the rise in pyrolysis temperature from 300 to 650 °C (Fig. 2). In this pyrolysis temperature range, CO₂ emissions appeared to be independent of hydrocarbon emissions suggesting that they do not correspond to the thermal cracking of the macromolecular network.

OHMM and OLMM isolated from the Rhynie chert were characterised by a TpkS2 of 481 °C and 504 °C, respectively (Table 1). In every type of kerogen, these pyrolysis temperatures are commonly assigned to OM that has been subjected to high thermal alteration. Consistent with high TpkS2 values, pyrolysis of OHMM and of OLMM released low amounts of HC as evidenced by HI of 46 HC/g TOC and 68 mg HC/g TOC, respectively. Together, high TpkS2 and low HI imply that OM from the Rhynie chert falls at the transition between the oil and gas windows registered by TpkS2 ranging between 470 °C and 505 °C in analogous Type III kerogens (Espitalié et al., 1986). Hydrocarbon release during Rock-Eval pyrolysis was higher in OLMM than in OHMM (Table 1). The S1 value for OLMM was about 320% higher than for OHMM (Table 1), which suggests that thermolabile OM was more abundant in OLMM than in OHMM. This is consistent with the fact that OLMM was not subjected to organic solvent extractions, which have been shown to dramatically reduce S1 values (Delvaux et al., 1990). Between 300 and 350 °C, no release of hydrocarbons was observed suggesting a virtual absence of residual heavy oil and/or of pyrobitumen in both OHMM and OLMM (Fig. 2; Clementz, 1979; Sanei et al., 2015). The S2 curves for OHMM

and OLMM differ at high pyrolysis temperature ($> 500\text{ }^{\circ}\text{C}$), at which point a broad shoulder is only observed in OLMM (Fig. 2). The occurrence of this thermorecalcitrant OM in OLMM mostly explains its higher HI, in the absence of solvent extractions and/or hydrochloric acid hydrolysis (Table 1). In turn, this suggests that standard acid maceration can lead to a modification of the macromolecular chemical structure in this ancient silicified OM by promoting a loss of hydrocarbonaceous moieties.

Determined on 15 random targets in both OHMM and OLMM, Raman spectra exhibited two broad D and G+D2 bands at around 1365 cm^{-1} and 1600 cm^{-1} in each preparation (Fig. 1). The D band presents a complex structure resulting from the presence of several sub-bands including here, the D4, D1, and D5 bands. In addition to the contribution of amorphous carbon, the D band is dominated by the D1 sub-band corresponding to the breathing mode of the sp^2 aromatic ring within polyaromatic clusters and is attributed to defects in these aromatic structures (Ferrari and Robertson, 2000). The D2 band corresponds to defects in aromatic structure but in contrast to the D1 band, does not include amorphous carbon. A distinct D2 peak (usually centered at about 1620 cm^{-1}) is mainly observed in highly mature OM (Kouketsu et al. 2014) and is not observed in OM studied here. Therefore, the G+D2 band is mostly related to the G band corresponding to in-plane C-C bond stretching in polyaromatic layers from thermally altered materials (Marshall and Marshall, 2014). As the height of the D1 band increases with temperature, the $I_{\text{D1}}/I_{\text{G+D2}}$ ratio is often used to probe the structural order of OM in the course of carbonization (Table 2; Wopenka and Pasteris, 1993; Quirico et al., 2005; Kouketsu et al., 2014; Delarue et al., 2016). In this study, OHMM and OLMM are characterized by $I_{\text{D1}}/I_{\text{G+D2}}$ ratios of 0.58 ± 0.02 and 0.52 ± 0.02 , respectively (Table 2). This result would imply a higher carbon structural order in OHMM compared to OLMM, which is in agreement with previous findings indicating that standard acid maceration can yield a rise in carbon structural order (Zhang et al., 2016).

The D band also exhibits two shoulders corresponding to the D4 (1285 cm⁻¹) and D5 (1445 cm⁻¹) bands (Fig. 1). The D4 peak is generally assigned to C–H bonds in aliphatics as C–H in aromatics does not seem to directly contribute to the D4 region (Ferralis et al., 2016). The D5 band indicates the presence of hydrocarbons trapped within the organic porosity and has been detected in a few oil and gas shale samples (Romero-Sarmiento et al., 2014; Rouzaud et al., 2015). In the current study, α_{D4} and α_{D5} slopes in Raman spectra were lower in OLMM than in OHMM, consistent with the more pronounced D4 and D5 shoulders in the Raman line of OLMM (Fig. 1; Table 2). However, the more pronounced D4 and D5 shoulders in OLMM were not associated with higher I_{D4}/I_{G+D2} and I_{D5}/I_{G+D2} ratios (Table 2). This could be explained by the higher carbon structural order in OHMM implying a bias in the determination of I_{D4}/I_{G+D2} and I_{D5}/I_{G+D2} ratios. The occurrence of more pronounced D4 and D5 shoulders in OLMM indicate that OLMM is indeed enriched in hydrocarbonaceous moieties, due to the C–H bonds in aliphatics and other entrapped hydrocarbons, relative to OHMM. The presence of entrapped hydrocarbons in the macromolecular network of OLMM likely explains the broad shoulder cracking at high temperature (> 500 °C) as revealed by Rock-Eval pyrolysis (Fig. 2). Both Raman spectroscopy and Rock-Eval pyrolysis suggest that standard acid maceration results in a loss of hydrocarbons in OM isolated from the Rhynie chert. As LMM does not involve the organic solvent extraction used in HMM, part of this hydrocarbon loss is likely due to solvent-extractible OM, which is reflected by the respective S1 values. In contrast to OHMM, OLMM did not involve extraction with HCl, which when mixed with HF has been demonstrated to favour hydrolysis leading to a loss of aliphatic CH and/or CH₂ functional groups in coal (Tekely et al., 1987). Even though our experimental design did not allow us to test the singular effect of HCl hydrolysis, such an effect can be hypothesized to partly explain the loss in aliphatic moieties with HMM treatment.

Although the Raman spectroscopy results support the notion that hydrocarbons are lost during HMM, it does not directly allow the extent of this loss to be determined. Following the relationship between H/C and HI (Espitalié et al., 1977), we determined that H/C atomic ratios were 0.53 and 0.57 in OHMM and OLMM, respectively (Table 1). Standard acid maceration therefore led to a reduction of at least about 7–8% of the hydrocarbon content of Rhynie OM. We use “at least” because chemical degradation of hydrocarbons occurring during the isolation of OLMM cannot be straightforwardly excluded. To evaluate this point, Raman spectra were also acquired on in situ OM from thin sections of Rhynie chert (Supplementary information). These in situ Raman spectra were acquired with another Raman spectroscope and on polished thin sections, which can modify the apparent carbon structural order by yielding an increase in the D band (Ammar et al., 2011; Maslova et al., 2012). These issues therefore prevent a straightforward comparison between Raman-derived ratios from in situ OM and from OLMM/OHMM. Nonetheless, the α_{D4} and α_{D5} parameters are independent of the height of the D band and can be used to track a potential effect of LMM procedure. In situ OM presents a mean α_{D4} and α_{D5} of about 31 ± 4 and -24 ± 23 , respectively ($n = 10$), indicating more pronounced D4 and D5 shoulders in the in situ OM compared to OLMM (Supplementary information). This suggests that simple treatment with HF and water also led to a slight loss in aliphatic content. In addition to these hydrocarbon losses related to acid maceration procedures, the estimated 7–8% loss in hydrocarbons can also be considered as minimum estimation because of thermolabile hydrocarbons (S1 parameter), which are not taken into account in the calculation of HI and were more abundant in OLMM than in OHMM.

Thermolabile hydrocarbons are tightly linked to soluble OM (Delvaux et al., 1990). However, this soluble OM in ancient sediments is often ignored because its syngeneity can be difficult to prove due to the potential for post-deposit contamination (Brocks et al., 2003a,

2003b; Derenne et al., 2008). However, silicified sediments can be seen as unconventional dual source/reservoir rocks. Indeed, rapid silicification drastically reduces porosity leading to a closed chemical system (Boyce et al., 2002; Ledevin et al., 2014). In such source/reservoir rocks, migration of bitumens was very restricted implying that bitumens and/or pyrobitumens coexisted in close proximity with residual kerogens (Vandenbroucke and Largeau, 2007). Hence, in ancient silicified sediments, bitumens and pyrobitumens can be considered syngenetic.

In contrast to soluble OM, insoluble OM is often considered less prone to late contamination (Derenne et al., 2008). Involving no chemical treatment or low-manipulation procedures, numerous studies have investigated the molecular structure of OM – at the scale of plant fossils – to assess the effects of fossilization or to depict plant evolution and affinities (Ewbank et al., 1996; Edwards et al., 1997; Abbott et al., 1998; Czaja et al., 2009; Quijada et al., 2016). Because a large proportion of the hydrocarbon content of these samples is preserved, these investigations minimized secondary hydrothermal and endolithic contaminations – which can also yield insoluble OM – by studying insoluble OM directly from plant fossils. To avoid contamination and to optimize the study of syngenetic hydrocarbons that can be degraded in the course of acid maceration, the development of in situ molecular micrometre-scale analyses techniques (e.g., time-of-flight secondary ion mass spectrometry and laser micropyrolysis) are of interest. Such techniques have the potential to directly probe the molecular structure of organic fossils independent of the soluble/insoluble operational definition. In addition to insightfully assessing chemical heterogeneity within macrofossils (Boyce et al., 2002; Abbott et al., 2018), in situ molecular micrometre-scale analyses also offers the possibility to study the molecular composition of micrometric to sub-micrometric diffuse particulate OM (Stout, 1993; Greenwood et al., 2001; Yoshioka and

Takeda, 2004; Silva et al., 2016). This includes remnants of microorganisms, such as organic-walled microfossils, which are essential organic components of the early geological record. In the specific context of silicified rocks – where silicification prevented migration of bitumens and favoured formation of pyrobitumen in the close vicinity of the residual kerogen – the use of in situ molecular micrometre-scale analyses offers promise in the investigation of the molecular composition of early life found within Archean silicified sediments. Indeed, the emergence of micrometre-scale *in situ* analyses have provided compelling evidence supporting an unexpected and wide chemical heterogeneity among Archean putative organic-walled microfossils (Delarue et al., 2017, 2020; Hickman-Lewis et al., 2020). Overlooked by bulk extraction approaches, this chemical heterogeneity defines a chemically-well preserved end product among the earliest traces of life. Therefore, the precise study of such geological archives requires further development of in situ molecular micrometre-scale analyses. Such techniques may pave the way to document biological and abiotic processes and affinities of the earliest putative remnants of life by: (i) focussing on chemically well preserved specimens, (ii) without the use of chemical extraction and maceration techniques, which can degrade aliphatic moieties preserved in sediments subjected to early and prompt silicification.

4. Conclusions

In this study, we investigated the impact of standard acid maceration on the chemical structure of silicified OM from the Lower Devonian Rhynie chert. Using Rock-Eval pyrolysis and Raman spectroscopy, we assessed the chemical structure of OM isolated by two different physical and chemical manipulation procedures. Results from Rock-Eval pyrolysis and Raman spectroscopy converged to demonstrate that the standard procedure of OM isolation led to a significant and substantial degradation of hydrocarbons. These chemical alterations indicate that a significant amount of molecular content is lost as a result of standard acid

maceration procedures, thus preventing a thorough examination of the molecular content of organic remnants in thermally altered silicified sediments.

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Figure captions

Fig. 1. (a) Typical Raman spectra measured on OHMM (black line) and OLMM (grey line).

Fig. 2. Emissions of (a) hydrocarbons and (b) CO₂ during Rock-Eval pyrolysis of OHMM (black line) and OLMM (grey line). The red curve indicates the pyrolysis temperature program. The green area in (b) indicates the temperature interval, for which CO₂ emissions are included in the calculation of OI.

Table 1: Rock-Eval parameters determined in OHMM and OLMM. *H/C atomic ratio was estimated using the relationship between H/C atomic ratio and HI ($\text{H/C} = 0.0017 \times \text{HI} + 0.453$) published by Espitalié et al. (1977).

	S1 (mg HC/g)	S2 (mg HC/g)	TpkS2 (°C)	TOC (%)	HI (mg HC/g TOC)	OI (mg CO₂/g TOC)	H/C atomic ratio*
OHMM	0.59	11.76	481	25.4	46	59	0.53
OLMM	2.48	18.83	504	27.5	68	15	0.57

Table 2: Raman-derived parameters (mean value $\pm$ S.D.) determined in OHMM and OLMM (n= 15; Wilcoxon rank test).

	I_{D1}/I_{G+D2}	I_{D4}/I_{G+D2}	I_{D5}/I_{G+D2}	α_{D4}	α_{D5}
OHMM	0.58 ± 0.02	0.38 ± 0.02	0.43 ± 0.04	359 ± 35	-288 ± 39
OLMM	0.52 ± 0.02	0.36 ± 0.02	0.31 ± 0.01	202 ± 37	-240 ± 28
<i>p</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001