1 2 3	Supportin	g information
4	Assessing biodegradation of roadway particles	
5	via complementary mass spectrometry and NMR analyses.	
6		
7	Laurie Calarnou ¹ , Mounir Traïkia ¹ , Martin Le	remboure ¹ , Lucie Malosse ² , Séverin Dronet ² ,
8	Anne-Marie Delort ¹ , Pascale Besse-Hoggan ¹ ,	Boris Eyheraguibel ¹
9 10	¹ Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut de Chimie (ICCF), F-63000 Clermont– Ferrand, France.	
11 12	² Manufacture Française des Pneumatiques MICHELIN, Centre de Technologies Ladoux, 63040 Clermont-Ferrand, France.	
13		
1/		
15	Table S1. Emerson medium composition (for 1L of deionized water)	
	Composition	Concentration
	K ₂ HPO ₄	1 g.L ⁻¹
	MgSO ₄	
	Soluble starch	<u> </u>
	V t E-two . t	0.5 g.L ⁻¹
	Yeast Extract	0.5 g.L ⁻¹ <u>15 g.L⁻¹</u> <u>2.5 g.L⁻¹</u> <u>20 g.L⁻¹</u>
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for	0.5 g.L ⁻¹ 15 g.L ⁻¹ 2.5 g.L ⁻¹ 20 g.L ⁻¹ 1L ultrapure water)
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition	0.5 g.L ⁻¹ 15 g.L ⁻¹ 2.5 g.L ⁻¹ 20 g.L ⁻¹ 1L ultrapure water) Concentration
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition Na ₂ HPO ₄ .12H ₂ O	0.5 g.L ⁻¹ 15 g.L ⁻¹ 2.5 g.L ⁻¹ 20 g.L ⁻¹ 1L ultrapure water) Concentration 3.8 g.L ⁻¹
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition Na2HPO4.12H2O KH2PO4	0.5 g.L ⁻¹ 15 g.L ⁻¹ 2.5 g.L ⁻¹ 20 g.L ⁻¹ 1L ultrapure water) Concentration 3.8 g.L ⁻¹ 1.8 g.L ⁻¹
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition Ma2HPO4.12H2O KH2PO4 MgSO4.7H2O	0.5 g.L ⁻¹ 15 g.L ⁻¹ 2.5 g.L ⁻¹ 20 g.L ⁻¹ 1L ultrapure water) Concentration 3.8 g.L ⁻¹ 1.8 g.L ⁻¹ 20 mg.L ⁻¹
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition Ma2HPO4.12H2O KH2PO4 MgSO4.7H2O Fe(NH4)2(SO4)2.6H2O	0.5 g.L^{-1} 15 g.L^{-1} 2.5 g.L^{-1} 20 g.L^{-1} $1\text{L ultrapure water)}$ $\frac{\text{Concentration}}{3.8 \text{ g.L}^{-1}}$ 1.8 g.L^{-1} 20 mg.L^{-1} 30 mg.L^{-1}
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition Mageodynamic and the second secon	0.5 g.L^{-1} 15 g.L^{-1} 2.5 g.L^{-1} 20 g.L^{-1} $1L \text{ ultrapure water}$ $1L \text{ ultrapure water}$ $1L \text{ ultrapure water}$ $1L \text{ ultrapure water}$ 20 g.L^{-1} 1.8 g.L^{-1} 20 mg.L^{-1} 30 mg.L^{-1} 10 mg.L^{-1}
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition Ma2HPO4.12H2O KH2PO4 MgSO4.7H2O Fe(NH4)2(SO4)2.6H2O CaCl2.2H2O NaCl	$\begin{array}{r} 0.5 \text{ g.L}^{-1} \\ 15 \text{ g.L}^{-1} \\ 2.5 \text{ g.L}^{-1} \\ 20 \text{ g.L}^{-1} \\ \end{array}$ $\begin{array}{r} 1L \text{ ultrapure water} \end{array}$ $\begin{array}{r} \hline \\ \hline $
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition Ma2HPO4.12H2O Ma2HPO4.12H2O KH2PO4 MgSO4.7H2O Fe(NH4)2(SO4)2.6H2O CaCl2.2H2O NH4Cl	$\begin{array}{c} 0.5 \text{ g.L}^{-1} \\ 15 \text{ g.L}^{-1} \\ 2.5 \text{ g.L}^{-1} \\ 20 \text{ g.L}^{-1} \\ \end{array}$ $\begin{array}{c} 1L \text{ ultrapure water} \end{array}$ $\begin{array}{c} 1L \text{ ultrapure water} \end{array}$ $\begin{array}{c} 20 \text{ mg.L}^{-1} \\ 1.8 \text{ g.L}^{-1} \\ 20 \text{ mg.L}^{-1} \\ 30 \text{ mg.L}^{-1} \\ 0.5 \text{ g.L}^{-1} \\ 0.5 \text{ g.L}^{-1} \\ \end{array}$
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition Ma2HPO4.12H2O KH2PO4 MgSO4.7H2O Fe(NH4)2(SO4)2.6H2O CaCl2.2H2O NH4Cl MnSO4.H2O	$\begin{array}{r} 0.5 \text{ g.L}^{-1} \\ 15 \text{ g.L}^{-1} \\ 2.5 \text{ g.L}^{-1} \\ 20 \text{ g.L}^{-1} \\ \end{array}$ 1L ultrapure water) $\begin{array}{r} \hline \\ \hline $
16 17	$\begin{tabular}{ c c c c c } \hline Yeast Extract \\ \hline Agar \\ \hline \hline Table S2. Mineral salt medium composition (for \\ \hline \hline Composition \\ \hline \hline Na_2HPO_4.12H_2O \\ \hline Na_2HPO_4.12H_2O \\ \hline KH_2PO_4 \\ \hline MgSO_4.7H_2O \\ \hline Fe(NH_4)_2(SO_4)_2.6H_2O \\ \hline CaCl_2.2H_2O \\ \hline NaCl \\ \hline NH_4Cl \\ \hline NH_4Cl \\ \hline MnSO_4.H_2O \\ \hline H_3BO_3 \\ \hline \end{tabular}$	$\begin{array}{c} 0.5 \text{ g.L}^{-1} \\ 15 \text{ g.L}^{-1} \\ 2.5 \text{ g.L}^{-1} \\ 20 \text{ g.L}^{-1} \end{array}$ $\begin{array}{c} 1L \text{ ultrapure water} \end{array}$ $\begin{array}{c} 1L \text{ ultrapure water} \end{array}$ $\begin{array}{c} 0.8 \text{ g.L}^{-1} \\ 1.8 \text{ g.L}^{-1} \\ 20 \text{ mg.L}^{-1} \\ 20 \text{ mg.L}^{-1} \\ 10 \text{ mg.L}^{-1} \\ 0.5 \text{ g.L}^{-1} \\ 18.5 \text{ mg.L}^{-1} \\ 20 \text{ mg.L}^{-1} \end{array}$
16 17	Yeast Extract Agar Table S2. Mineral salt medium composition (for Composition Na2HPO4.12H2O KH2PO4 MgSO4.7H2O Fe(NH4)2(SO4)2.6H2O CaCl2.2H2O NaCl NH4Cl MnSO4.H2O H3BO3 ZnSO4.7H2O	$\begin{array}{c} 0.5 \text{ g.L}^{-1} \\ 15 \text{ g.L}^{-1} \\ 2.5 \text{ g.L}^{-1} \\ 20 \text{ g.L}^{-1} \\ \end{array}$ $\begin{array}{c} 1L \text{ ultrapure water} \end{array}$ $\begin{array}{c} 1L \text{ ultrapure water} \end{array}$ $\begin{array}{c} 0.8 \text{ g.L}^{-1} \\ 1.8 \text{ g.L}^{-1} \\ 20 \text{ mg.L}^{-1} \\ 1.8 \text{ g.L}^{-1} \\ 30 \text{ mg.L}^{-1} \\ 10 \text{ mg.L}^{-1} \\ 0.5 \text{ g.L}^{-1} \\ 10 \text{ mg.L}^{-1} \\ 0.5 \text{ g.L}^{-1} \\ 18.5 \text{ mg.L}^{-1} \\ 20 \text{ mg.L}^{-1} \\ 18.5 \text{ mg.L}^{-1} \\ 2.5 \text{ mg.L}^{-1} \end{array}$
16 17	$\begin{tabular}{ c c c c c } \hline Yeast Extract \\ \hline Agar \\ \hline \hline Table S2. Mineral salt medium composition (for \\ \hline \hline Composition \\ \hline \hline Na_2HPO_4.12H_2O \\ \hline Na_2HPO_4.12H_2O \\ \hline \hline MgSO_4.7H_2O \\ \hline Fe(NH_4)_2(SO_4)_2.6H_2O \\ \hline CaCl_2.2H_2O \\ \hline NaCl \\ \hline NH_4Cl \\ \hline MnSO_4.H_2O \\ \hline H_3BO_3 \\ \hline ZnSO_4.7H_2O \\ \hline Na_2MoO_4.2H_2O \\ \hline \hline Na_2MoO_4.2H_2O \\ \hline \hline \end{array}$	$\begin{array}{c} 0.5 \text{ g.L}^{-1} \\ 15 \text{ g.L}^{-1} \\ 2.5 \text{ g.L}^{-1} \\ 20 \text{ g.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 1L \text{ ultrapure water} \end{array}$ $\begin{array}{c} 1L \text{ ultrapure water} \end{array}$ $\begin{array}{c} 0.8 \text{ g.L}^{-1} \\ 1.8 \text{ g.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 20 \text{ mg.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 0.0 \text{ mg.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 0.5 \text{ g.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 0.3 \text{ g.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 0.3 \text{ g.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 0.3 \text{ g.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 20 \text{ mg.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 0.5 \text{ g.L}^{-1} \\ \hline \end{array}$ $\begin{array}{c} 20 \text{ mg.L}^{-1} \\ \hline \end{array}$
16 17	Yeast ExtractAgarTable S2. Mineral salt medium composition (forCompositionNa $_2$ HPO $_4$.12H $_2$ OKH $_2$ PO $_4$ MgSO $_4$.7H $_2$ OFe(NH $_4$) $_2$ (SO $_4$) $_2$.6H $_2$ OCaCl $_2$.2H $_2$ ONaClNH $_4$ ClMnSO $_4$.H $_2$ OHaBO $_3$ ZnSO $_4$.7H $_2$ ONa $_2$ MoO $_4$.2H $_2$ OCoSO $_4$	$\begin{array}{c} 0.5 \text{ g.L}^{-1} \\ 15 \text{ g.L}^{-1} \\ 2.5 \text{ g.L}^{-1} \\ 20 \text{ g.L}^{-1} \end{array}$ $\begin{array}{c} 11 \text{ Ultrapure water} \end{array}$ $\begin{array}{c} 11 \text{ Ultrapure water} \end{array}$ $\begin{array}{c} 11 \text{ Ultrapure water} \end{array}$ $\begin{array}{c} 20 \text{ g.L}^{-1} \\ 1.8 \text{ g.L}^{-1} \\ 20 \text{ mg.L}^{-1} \\ 20 \text{ mg.L}^{-1} \\ 10 \text{ mg.L}^{-1} \\ 0.5 \text{ g.L}^{-1} \\ 0.3 \text{ g.L}^{-1} \\ 18.5 \text{ mg.L}^{-1} \\ 20 \text{ mg.L}^{-1} \\ 20 \text{ mg.L}^{-1} \\ 2.5 \text{ mg.L}^{-1} \\ 2.5 \text{ mg.L}^{-1} \\ 18 \text{ g.g.L}^{-1} \\ 2.5 \text{ mg.L}^{-1} \\ 10 \text{ g.g.L}^{-1} \\ 2.5 \text{ mg.L}^{-1} \\ 10 \text{ g.g.L}^{-1} \\ 10 g.g.L$





21 Fig. S1. Nozzles used for collection of RP





24

Fig. S2. ¹H NMR spectra of RP extracts obtained after 21 days of contact under agitation. (A)
water extract (D₂O); (B) methanol extract (methanol-d₄); (C) acetone extract (acetone-d₆); (D)
chloroform extract (CDCl₃)

28 3.1.1. Analysis of the leachate profiles and SBR by NMR spectroscopy

29 Multi-dimensional analysis were performed to validate peak assignment. ¹H NMR analysis of the oil entering the target tire formulation showed a signal at $\delta_{\rm H}$ 5.2–5.5 ppm that can be 30 assigned to the =CH ethylenic protons of the unsaturated fatty acids, revealing an overlapping 31 32 region with the signal of SBR 1,4-butadiene moieties (Region B, Fig. 2). The other signals of 33 the RP extract at $\delta_{\rm H} \sim 4.8-5.05$ ppm and 5.5–5.7 ppm (Fig. 2; Regions C and A, respectively) 34 could be assigned to =CH₂ and =CH ethylenic protons of 1,2-butadiene moieties of SBR, respectively, and the signal in region D ($\delta_{\rm H} \sim 4.1$ -4.3 ppm) to protons of oil unsaturated fatty 35 acids. 36

- 37 2D HSQC spectra were performed on chloroform RP extract, oil and SBR standard to highlight
- 38 potential contribution of other compounds. Comparison of the aromatic region ($\delta_H \sim 7.0-7.2$
- 39 ppm) on the 2D HSQC spectra revealed the presence of several other signals which cannot be
- 40 assigned to SBR (Fig. S2), making this region unreliable for SBR monitoring in RP extracts.



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42 **Fig. S3.** Liquid state HSQC NMR spectra of SBR in CDCl₃ (blue) and 21-day chloroform 43 extract of RP (black) (δ H ~ 6.90–7.45 ppm)

44

In the butadiene proton region ($\delta_{\rm H} \sim 4.8-5.7$ ppm), the signals corresponding to oil unsaturated fatty acids and 1,4-butadiene protons cannot be distinguished by 2D HSQC either (Region B). On the contrary, the regions A and C, assigned to 1,2-butadiene ethylenic protons, were validated by 2D HSQC as any other contribution was observed (Fig. S3).



Fig. S4. Liquid state HSQC NMR spectra of SBR (blue) and oil (red) in CDCl₃ and 21-day chloroform extract of RP (black) (δ H ~ 4.7–5.8 ppm). For a better understanding and vizualization, the spectra of SBR and oil have been shifted up.



Fig. S5. ¹H NMR spectra of RP extracts (CDCl₃) of abiotic (red) and *Rhodococcus ruber* ATCC

57 29672 (black) incubation after 14 days ($^{\delta}$ H ~ 4.1–5.7 ppm)