Diversity of isoprene-degrading bacteria in phyllosphere and soil communities from a high isoprene-emitting environment: a Malaysian oil palm plantation

Ornella Carrión[1‡*], Lisa Gibson[1‡], Dafydd M.O. Elias[2], Niall P. McNamara[2], Theo A. van Alen[3], Huub J.M. Op den Camp[3], Christina Vimala Supramaniam[4], Terry J. McGenity[5], J. Colin Murrell[1*]

[1]School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK

[2]UK Centre of Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK

[3]Department of Microbiology, Faculty of Science, IWWR, Radboud University Nijmegen, Heyendaalseweg 135, NL-6525 AJ Nijmegen, The Netherlands

[4]School of Biosciences, Nottingham Centre of Sustainable Palm Oil, University of Nottingham-Malaysia, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia

[5]School of Life Sciences, University of Essex, Colchester, UK

[‡]These authors contributed equally to this work

*Corresponding authors:

J Colin Murrell, [1]School of Environmental Sciences, University of East Anglia
Norwich Research Park, NR4 7TJ, UK
E-mail: j.c.murrell@uea.ac.uk
Tel: (+44) 01603 592959

Ornella Carrión, [1]School of Environmental Sciences, University of East Anglia
Norwich Research Park, NR4 7TJ, UK
E-mail: o.carrion-fonseca@uea.ac.uk
Tel: (+44) 01603 592239

Abstract

Background: Isoprene is the most abundantly produced biogenic volatile organic compound (BVOC) on Earth, with annual global emissions almost equal to those of methane. Despite its importance in atmospheric chemistry and climate, little is known about the biological degradation of isoprene in the environment. The largest source of isoprene is terrestrial plants, and oil palms, the cultivation of which is expanding rapidly, are among the highest isoprene-producing trees.

Results: DNA stable isotope probing (DNA-SIP) to study the microbial isoprene-degrading community associated with oil palm trees revealed novel genera of isoprene-utilising bacteria including *Novosphingobium, Pelomonas, Rhodoblastus*, *Sphingomonas* and *Zoogloea* in both oil palm soils and on leaves. Amplicon sequencing of *isoA* genes, which encode the α-subunit of the isoprene monooxygenase (IsoMO), a key enzyme in isoprene metabolism, confirmed that oil palm trees harbour a novel diversity of *isoA* sequences. In addition, metagenome assembled genomes (MAGs) were reconstructed from oil palm soil and leaf metagenomes and putative isoprene degradation genes were identified. Analysis of unenriched metagenomes showed that *isoA*-containing bacteria are more abundant in soils than in the oil palm phyllosphere.

Conclusion: This study greatly expands the known diversity of bacteria that can metabolise isoprene and contributes to a better understanding of the biological degradation of this important but neglected climate-active gas.

Keywords: Isoprene, climate, isoprene monooxygenase, DNA stable isotope probing, oil palm.

Background

Isoprene (2-methyl-1, 3-butadiene), with atmospheric emissions of 400-600 $Tg \cdot y^{-1}$, is the most abundantly produced biogenic volatile compound (BVOC) on Earth. This is approximately one-third of the total volatile organic compounds (VOCs) emissions and almost equal to global annual emissions of methane [1, 2]. Due to its volatile nature and high reactivity, isoprene plays a complex role in atmospheric chemistry and hence, climate. In pristine environments with low levels of nitrogen oxides (NOx), isoprene reacts with hydroxyl radicals (OH) and reduces the oxidising capacity of the atmosphere, which in turn increases the lifetime of greenhouse gases such as methane. However, when NOx are present at high levels, as commonly found in urban areas, they react with isoprene to form nitrogen dioxide (NO_2) and increase the tropospheric levels of ozone, which has detrimental impacts on air quality, human health and impedes progress towards many of the UN sustainable development goals [3-6]. Conversely, the products of isoprene oxidation can form secondary aerosols and act as cloud condensation nuclei, resulting in a global cooling effect [7].

While there are some industrial sources of isoprene (0.8 $Tg \cdot y^{-1}$), primarily from the production of synthetic rubber [8], the vast majority of isoprene emissions (~90%) originate from terrestrial plants [9, 10], with small contributions from marine algae (0.1-12 $Tg \cdot y^{-1}$), bacteria, fungi and animals [9, 11-16]. The enzyme responsible for isoprene production in plants is isoprene synthase, the presence and activity of which can vary significantly even between closely related species [17-20]. In isoprene-emitting plants, isoprene is produced in the chloroplast via the methyl-erythritol 4-phosphate (MEP) pathway [21]. Isoprene synthase is responsible for converting dimethylallyl diphosphate (DMAPP) to isoprene. Despite the fact that up to 2% of the carbon fixed by isoprene-emitting plants contributes to the synthesis of isoprene [22-23], its role in

plants is not fully understood. It has been reported that isoprene improves the resilience of plants to oxidative, thermal and biotic stresses [23-26]. However, the molecular mechanisms behind these processes have not yet been fully elucidated. In addition, it has been recently suggested that isoprene may play a role in regulating gene expression in plants [27].

While the production and atmospheric fate of isoprene has been well studied, biological consumption in the isoprene biogeochemical cycle remains relatively unexplored. Field chamber and continuous-flow studies have shown that soils are a biological sink for isoprene at environmentally relevant concentrations [28-30]. Several bacterial strains capable of growing on isoprene as a sole carbon and energy source have been isolated from soils, phyllosphere and aquatic environments (reviewed in [31]). Most of these strains are Gram-positive Actinobacteria, although more recent studies have led to the isolation of Gram-negative Proteobacteria expanding the known diversity of isoprene-degrading bacteria [32]. All characterised isoprene-utilising microorganisms contain six genes (*isoABCDEF*) that encode the isoprene monooxygenase (IsoMO) enzyme, which catalyses the first step in the isoprene degradation pathway. Adjacent genes *isoGHIJ* encode enzymes involved in the subsequent steps of isoprene metabolism [33]. The IsoMO belongs to the soluble diiron monooxygenase (SDIMO) family [34] and the α-subunit contains the diiron centre at the putative active site. The gene encoding this IsoMO α-subunit, *isoA*, is highly conserved in isoprene-utilising bacteria and is an excellent marker gene for isoprene degraders [35, 36]. The development of probes targeting *isoA* has been a successful approach to investigate the distribution, diversity and abundance of isoprene degraders in several environments, including oil palm soils and leaves [35]. However, it is important to combine the use of *isoA* probes with other cultivation-

103 independent techniques such as DNA stable isotope probing (DNA-SIP) [37] to
104 investigate the diversity of active isoprene degraders in the environment and to better
105 assess the role that microbes play in the biogeochemical cycle of isoprene. Indeed,
106 previous DNA-SIP experiments with ^{13}C-labelled isoprene have led to the identification
107 of novel genera of isoprene degraders in phyllosphere and soil environments, such as
108 *Sphingopyxis, Ramlibacter* and *Variovorax* [32, 38]. In turn, the sequencing
109 information provided by these DNA-SIP experiments has allowed the design of
110 targeted cultivation strategies that resulted in the isolation of representative strains of
111 these novel genera of isoprene degraders [32], which now can be used as model
112 microorganisms to study how isoprene metabolism is regulated.

113 The oil palm tree (*Elaeis guineensis*) is one of the highest isoprene-producing trees,
114 with estimated emissions of 175 $\mu g \cdot g^{-1}$ (dry leaves)$\cdot h^{-1}$ [39]. Oil palm is a major crop
115 across South East Asia and is the source of 30% of the world's vegetable oil [40], and
116 in countries such as Malaysia it covers >85% of total agricultural land, with an ongoing
117 annual land usage increase of 6.9% attributed solely to oil palm cultivation [40, 41].
118 Therefore, the vast expansion of a single crop that emits such high amounts of
119 isoprene have raised serious concerns about the impact of oil palm plantations on air
120 quality [42]. Here, we combine cultivation-dependent techniques with DNA-SIP, *isoA*
121 and 16S rRNA gene amplicon sequencing, and focussed metagenomics, to study the
122 isoprene-degrading microbial communities associated with oil palm trees in a
123 Malaysian plantation, both from the phyllosphere and from the soil nearby.

124

125

126

Results and Discussion

Identification of active isoprene-degrading bacteria using DNA-SIP

Diversity of bacteria from soils in the vicinity of oil palm trees

Analysis of 16S rRNA gene amplicon sequences showed that the unenriched bacterial community from soils in the vicinity of oil palm trees was very similar across replicates, confirming that extraction and handling procedures were consistent (Fig S1 and Fig S2 show the relative abundance (RA) of 16S rRNA genes in these environmental samples). The unenriched soil microbial community (S T0) was mainly composed of Proteobacteria (40.8 ± 0.5% RA), Actinobacteria (13.1 ± 0.7%), Bacteroidetes (11.2 ± 1.4%) and Acidobacteria (10.8 ± 0.6%, Fig S1), all of which are dominant phyla in soils [43-46]. The most abundant genera were *Rhodoplanes* (5.9 ± 0.1%) and *Flavobacterium* (4.0 ± 0.9%; Fig S2).

Soils were then enriched with ^{13}C-isoprene to identify the active isoprene degraders in this environment through DNA-SIP (see Methods). Sequencing of the 16S rRNA genes of the ^{13}C-heavy fractions showed that, although there was considerable inter-sample variability, *Rhodoblastus* (10.2-33.7%) and *Pelomonas* (14.2-54.9%) were highly enriched in all soil replicates (S 13C H; Fig 1). *Novosphingobium* was one of the major genera labelled in the ^{13}C-heavy fractions of replicates 2 (S 13C H R2) and 3 (S 13C H R3) representing 47.8% and 24.5%, respectively. Finally, *Sphingomonas* dominated the isoprene-degrading community of replicate 3 (S 13C H R3) with a RA of 42.4% (Fig 1). These four genera had 19 to 90-fold higher RA in ^{13}C-heavy (S 13C H) than in the ^{13}C-light (S 13C L) soil fractions, and constituted 28.7-40.2% of the total microbial community of the unfractionated soils incubated with ^{13}C-isoprene (13C UF;

Fig S2), which strongly suggest that they are active isoprene degraders. As expected, *Novosphingobium, Pelomonas, Sphingomonas* and *Rhodoblastus* also dominated the ^{12}C-isoprene-incubated microbial community (S 12C L) and each genus had a very similar RA to those of the unfractionated ^{13}C-samples (S 13C UF; Fig S2).

Previous DNA-SIP experiments and cultivation-dependent studies have identified members of *Sphingomonadaceae* (*Sphingopyxis*) and *Comamonadaceae* (*Ramlibacter* and *Variovorax*) as isoprene degraders with a functional IsoMO [32, 36, 38]. However, this is the first evidence that other genera of these families such as *Sphingomonas, Novosphingobium* and *Pelomonas* are likely to be able to metabolise isoprene. In addition, *Rhodoblastus* is the first member of the *Beijerinckiaceae* family to be implicated in isoprene degradation. Therefore, it will be interesting to attempt to isolate representative strains of this genus in future studies in order to confirm this ability.

DNA-SIP and 16S rRNA gene amplicon sequencing showed that the oil palm soil harbours a distinct isoprene-degrading bacterial community from soils beneath temperate trees that emit high levels of isoprene, such as willow. DNA-SIP experiments using willow soil incubated with ^{13}C-labelled isoprene identified *Rhodococcus, Ramlibacter* and *Variovorax* as the major genera labelled in the ^{13}C-heavy fractions [32]. However, these genera represented <1% of the ^{13}C-heavy (S 13C H) fractions from oil palm soil. Also, other well-characterised isoprene-degrading microorganisms, such as *Gordonia, Nocardioides, Mycobacterium* and *Sphingopyxis* species [32, 47, 48], constituted only a small part of the isoprene-degrading community (<1%) from soils taken from the vicinity of oil palm trees.

Both unenriched samples and heavy DNA fractions from these soil incubations were also subjected to metagenomic sequencing. Community composition of raw reads was assessed with MetaPhlAn2 [49]. As MetaPhlAn2 uses a range of clade-specific marker genes to assess the phylogeny of the metagenomics reads, results differed slightly from those obtained using 16S rRNA gene amplicon sequencing analysis. According to the phylogenetic analysis of the soil metagenomes, the unenriched soil (S T0) community was dominated by Proteobacteria (81.5%), Actinobacteria (7.8%) and Acidobacteria (6.3%; Fig S1), confirming the results obtained by the analysis of the 16S rRNA gene amplicon sequencing data. The most abundant bacteria in the unenriched soils that could be classified at the genus level belonged to *Cupriavidus* (14.2%), followed by *Pseudogulbenkiania* (13.2%) and *Burkholderia* (11.7%; Fig 1).

Metagenomic sequencing revealed that ^{13}C-heavy fractions from replicates 1 and 2 (S 13C H R1-2) were dominated by *Thiomonas* (34.5%) and *Gordonia* (18.2%), whereas replicate 3 (S 13C H R3) had a higher abundance of *Gordonia* (47%) and *Sphingobium* (21.8%; Fig 1). However, these genera represented <1% of the ^{13}C-heavy fractions in the 16S rRNA gene amplicon sequencing data. It is not surprising to find members of *Gordonia* dominating the isoprene-degrading community, since strains from this genus have been shown to contain a complete isoprene degradation gene cluster [48]. However, this study provides the first evidence that *Thiomonas* and *Sphingobium* species may be also able to catabolise isoprene.

Diversity of bacteria from the phyllosphere of oil palm trees

The bacterial community of unenriched oil palm leaf (L T0) samples was dominated by Proteobacteria (74.5 ± 0.3%, Fig S1), which is not surprising since Proteobacteria

have been found to be the most abundant phylum in the phyllosphere of several plant species [50-53]. Firmicutes also constituted a major component of the unenriched bacterial community from oil palm leaves (22.1 ± 0.2%, Fig S1), as has been reported for some trees and agricultural plants [51, 52, 54-55]. The most abundant genera in the oil palm phyllosphere were *Acinetobacter* (26.4 ± 0.7%), followed by *Clostridium* (22.0 ± 0.2%) and *Enterobacter* (11.6 ± 0.2%; Fig 2).

The 16S rRNA gene amplicon sequencing data showed that the diversity of the isoprene-degrading community of the samples incubated with ^{13}C-isoprene (L 13C H) was highly consistent between replicates, with *Gordonia* (51.4 ± 9.4%) and *Zoogloea* (12.3 ± 2.2%) being the most abundant genera (Fig 2). The RA of *Gordonia* and *Zoogloea* was 84.9 and 58.2-fold higher in the ^{13}C-heavy (L 13C H) compared to the ^{13}C-light (L 13C L) fraction, respectively (Fig S3), indicating that they are active isoprene degraders. In addition, these two genera constituted 10.8% of the total microbial community of the ^{13}C-unfractionated (L 13C UF) samples and, as expected, were also highly abundant in the ^{12}C-isoprene-incubated (L 12C L) microbial community (13.6%; Fig S3).

Strains of *Gordonia* that grow on isoprene as sole carbon and energy source have been isolated previously from leaves of an oil palm tree in the Palm House of Kew Gardens, London [32]. However, although a number of SIP experiments with ^{13}C-isoprene have been performed with samples from a wide range of environments, including the phyllosphere, estuaries and soils, no members of the *Zoogloea* genus or the order *Rhodocyclales* have been identified as active isoprene degraders [32, 36, 38, 48]. Here, the identification of *Zoogloea* as an isoprene degrader indicates that the variety of microorganisms able to metabolise this important climate-active gas is greater than previously known.

224 *Rhizobium* also had a relatively high RA (8.5 ± 2.2%) in all replicates from ^{13}C-heavy
225 fractions (L 13C H) compared to the unenriched (L T0) samples (Fig 2). However, its
226 RA was 2.2-fold higher in the ^{13}C-heavy (L 13C H) than the ^{13}C-light (L 13C L)
227 fractions, which is the same ratio observed between the ^{12}C-heavy (L 12C H) and the
228 ^{12}C-light (L 12C L) fractions (Fig S3). Therefore, based on these data, and since no
229 strains of this genus have been isolated from this environment to corroborate its ability
230 to degrade isoprene, *Rhizobium* spp. cannot be yet confirmed as isoprene degraders.

231 Previous SIP experiments exploring the phyllosphere of other high isoprene-emitting
232 trees from temperate regions, such as poplar, identified *Rhodococcus* and *Variovorax*
233 as the major players in isoprene degradation [38]. However, in our experiment, the
234 phyllosphere from tropical oil palm trees yielded a distinct profile of active isoprene
235 degraders, with *Gordonia* and *Zoogloea* being the main genera enriched in the ^{13}C-
236 heavy fractions and *Rhodococcus* and *Variovorax* showing a low RA (2.1 ± 0.4% and
237 <1%, respectively). The RA of other well-characterised isoprene degraders such as
238 *Sphingopyxis, Ramlibacter, Nocardioides* or *Mycobacterium* [32, 48] also represented
239 <1% of the labelled bacterial community from oil palm leaves (L 13C H).

240 Phylogenetic analysis of the unenriched leaf (L T0) metagenomes confirmed that the
241 unenriched bacterial community of the oil palm phyllosphere was overwhelmingly
242 dominated by Proteobacteria (99.1% RA; Fig S1). At the genus level, metagenomics
243 analysis also supported the 16S rRNA gene amplicon sequencing data, since
244 *Acinetobacter* (40.8% RA) and *Enterobacter* (12.7% RA) were highly abundant in the
245 unenriched phyllosphere community, together with *Pantoea* (15.5% RA; Fig 2).

246 Metagenomic data showed that *Gordonia* constituted 93.7% of the isoprene-degrading
247 community of oil palm leaves (L 13C H R1-3; Fig 2), in accordance to the 16S rRNA

gene amplicon sequencing results. However, no *Zoogloea* sequences were identified in the ^{13}C-heavy fractions in the metagenomic analysis probably due to the different approach that MetaPhlAn2 uses to assign the phylogeny of the reads compared to the 16S rRNA gene amplicon analysis.

Comparison of isoprene degraders from soils and phyllosphere of oil palm trees

Studying the microbial diversity associated with plants is an essential step to understand host-microbiome interactions. However, only a few studies comparing microbial communities of phyllosphere and soils associated with the same plant species have been conducted to date (e.g. [54, 56-57]). Here we show that the unenriched bacterial communities from oil palm soils (S T0) and leaves (L T0) are distinct even at the phylum level (Fig S1), as reported for other plant species [54], although both soil and leaves are dominated by Proteobacteria. The 16S rRNA gene amplicon sequencing data also revealed that the active isoprene-degrading bacteria from soil samples were phylogenetically more diverse than those from the oil palm phyllosphere (see above).

When comparing the unenriched soil (S T0) and leaf (L T0) communities, it is interesting to note that although each major player in isoprene degradation was present in these contrasting environments at similar RA (Table S1), they responded differently to isoprene enrichment (S 13C H and L 13C H). This suggests that the physiochemical conditions and/or interactions with other groups of microorganisms shapes the composition of the isoprene-degrading community in a particular environment.

In addition, unenriched soil and leaf oil palm metagenomes (S T0 and L T0) were analysed for the presence and relative abundance of *isoA* genes. Metagenomic data showed that *isoA*-containing bacteria were 5-fold more abundant in soil samples (1% of Bacteria) than in the phyllosphere samples (0.2% of Bacteria). Metagenomes obtained in previous studies of unenriched samples from high isoprene-emitting trees from temperate regions, such as poplar [38] and willow [32], were also analysed for comparison. Results showed that 0.7% of Bacteria from soil beneath a willow tree and 0.02% of Bacteria from poplar leaves contained *isoA* genes. These data, though sparse, showed the same trend observed in the oil palm environment, with soils containing greater numbers of bacteria with the genetic potential to degrade isoprene than the phyllosphere. This finding is surprising considering the greater availability of isoprene in the canopy than at ground-level [58] and indicates that soils could be a more important sink for isoprene than previously thought.

Recovery of metagenome assembled genomes (MAGs)

Assembled contigs from soil and leaf metagenomes were used to reconstruct metagenome assembled genomes (MAGs) using MaxBin2 [59]. A total of 20 MAGs from soil and 52 from leaf samples were obtained (Table S2). From these, two MAGs from soils and three from leaf ^{13}C-heavy DNA metagenomes with >75% completeness and <10% contamination contained genes encoding homologous polypeptides to IsoABCDEF (E<1e-40). MAGs containing IsoMO-encoding genes from soil incubations were taxonomically classified as *Novosphingobium* and *Rhizobiales,* and leaf MAGs were classified as *Gordonia*, *Zoogloeaceae* and *Ralstonia* (Table S3).

The *Novosphingobium* soil-associated MAG contained the full isoprene degradation gene cluster (*isoABCDEFGHIJ*) on a single contig along with *aldH1*, which encodes an aldehyde dehydrogenase [33]. However, no further accessory genes were recovered (Fig 3). The products of these genes shared 76.2-100% amino acid identity (Table S4) with the corresponding polypeptides from *Sphingopyxis* sp. OPL5, a *Sphingomonadales* strain isolated from oil palm [32]. When the diversity and abundance of *isoA* genes in the ^{13}C-heavy DNA fractions from soil samples were analysed by *isoA* amplicon sequencing, two amplicon sequence variants (ASVs), ASV44 and ASV11, were identified, closely related to the IsoA from the *Novosphingobium* MAG (>99% amino acid identity; Table S5). These two ASVs represented 7.9% of the *isoA* genes of the ^{13}C-heavy DNA fraction from replicate 2 and 11% from replicate 3, respectively (Fig 4).

A *Rhizobiales* MAG was also reconstructed from ^{13}C-heavy DNA soil samples. Although this MAG had a high completeness (97.6%) and low contamination (2.5%), it showed a high strain heterogeneity (79%; Table S3), indicating that the MAG originated from DNA from one or more closely related microorganisms, thus making identification at a higher resolution difficult. Despite this, a complete isoprene degradation gene cluster (*isoABCDEFGHIJ*) plus *aldH1* were located in a single contig (Fig 3). When these genes were translated, they exhibited an amino acid identity of 54.8-84.9% to the homologous proteins from *Sphingopyxis* sp. OPL5, except for *isoD*, the product of which was more closely related to IsoD from *Ramlibacter* sp. WS9 [32] (Table S4). *isoA* gene amplicon sequencing analysis revealed that the ASVs closely related to the IsoA from the *Rhizobiales* MAG (>71% amino acid identity; Table S5) dominated the *isoA*-containing bacterial community from all ^{13}C-heavy DNA soil replicates, comprising a total of 78 ± 6.8% of the *isoA* genes in these samples (Fig 4).

The *Gordonia* phyllosphere MAG was identified to the species level as *Gordonia polyisoprenovorans* i37 [48] (average nucleotide identity; ANI: 96.7%) and contained all IsoMO-encoding genes *isoABCDEF*, along with downstream genes *isoGHIJ* (Fig 3). This MAG also contained *aldH2, CoA-DSR* and *gshB*, which are accessory genes often found within the isoprene degradation gene cluster in Gram-positive bacteria and encode an aldehyde dehydrogenase, a CoA-disulfide reductase and a glutathione synthetase, respectively [33]. All the genes associated with the isoprene degradation pathway recovered in the *Gordonia* MAG encoded polypeptides that shared >94% amino acid identity to the corresponding proteins from *Gordonia polyisoprenovorans* i37, except for IsoB (82.1%) and AldH1 (83%; Table S4). When the ^{13}C-heavy DNA fractions from leaf samples were analysed by *isoA* amplicon sequencing, several ASVs with a high percentage of amino acid identity to the IsoA from the *Gordonia* MAG (>93%; Table S5) were recovered. However, ASV1, which showed 100% amino acid identity to IsoA from the *Gordonia* MAG, overwhelmingly dominated the *isoA*-containing bacterial community, representing 91.9 ± 7.3% of the *isoA* genes in these samples (Fig 4).

The remaining two phyllosphere MAGs, *Zoogloeaceae* and *Ralstonia*, contained genes that encoded homologous polypeptides to IsoABCDEF, although they showed a low amino acid identity to the corresponding IsoMO proteins from well-characterised isoprene degraders (34.1-52.5%; Table S4). In addition, no gene homologues to *isoGHIJ* were recovered from these MAGs (Fig 3). While the absence of homologues to *isoGHIJ* and the low sequence identity to IsoABCDEF indicates that these bacteria may harbour a novel isoprene degradation pathway, especially considering that these MAGs were recovered from ^{13}C-heavy DNA fractions metagenomes, we cannot be absolutely certain that they are from *bona fide* isoprene degraders. These *isoA*-like

sequences were not identified by the *isoA* amplicon analysis, suggesting that the relatively high number of mismatches with the *isoA* primers prevented successful PCR amplification. Therefore, further targeted isolations of these bacteria and/or expression of these *isoABCDEF* genes in a heterologous host [38] are required to establish that these are genuine isoprene degraders.

Finally, if *Zoogloeaceae* and *Ralstonia* microorganisms could be confirmed as *bona fide* isoprene degraders, the abundance of bacteria with the potential to metabolise isoprene would increase from 1% to 2% in oil palm soil and from 0.1% to 0.2% in phyllosphere unenriched samples. Similarly, the RA of *isoA*-containing bacteria would also increase in both willow soil (from 0.7% to 1.3% of Bacteria) and poplar leaf (from 0.02% to 0.05% of Bacteria) unenriched samples, indicating that isoprene degraders could be more abundant in the environment than previously thought. However, more samples from contrasting ecosystems need to be explored to support this hypothesis.

Isolation and characterisation of *Variovorax* sp. OPL2.2

Cultures set up using material from the DNA-SIP experiments from soils and leaves were subcultured three times at 2-week intervals with 25 ppmv isoprene before plating onto minimal medium with isoprene as sole carbon source (see Methods). A strain belonging to the genus *Variovorax* isolated from leaf enrichments, was able to grow on isoprene as sole carbon and energy source (Fig S4).

Although DNA-SIP experiments have shown that *Variovorax* plays an important role in isoprene degradation in the phyllosphere [38], *Variovorax* sp. OPL2.2 is the first strain of this genus isolated from a tropical environment. When genomic DNA of OPL2.2 was screened for *isoA*, it yielded a PCR product which had a translated

sequence with 99.4% amino acid identity to IsoA from *Variovorax* sp. WS11, an isoprene degrader isolated from willow soil [32].

The genome of this new isolate from oil palm, *Variovorax* sp. OPL2.2, was sequenced using Illumina and Nanopore technologies to confirm that it contained a full isoprene degradation gene cluster. Assembly of both Illumina and Nanopore reads and downstream analysis with CheckM [60] revealed that the *Variovorax* sp. OPL2.2 genome was comprised of 50 contigs totalling 8.5 Mbp and had a 98.4% completeness, 1.1% contamination and a GC content of 67.4%. Finally, after automatic annotation by Prokka [61], 8,200 predicted coding sequences were found in the *Variovorax* sp. OPL2.2 genome.

Genome analysis confirmed that *Variovorax* sp. OPL2.2 contained *isoABCDEF* encoding IsoMO. *isoGHIJ* and *aldH1* genes, which are involved in the subsequent steps of isoprene metabolism, were located upstream *isoABCDEF* in an identical layout to those of many *bona fide* isoprene-degrading strains [38] (Fig 3). *garB*, which encodes a glutathione disulfide reductase, was also located in the same gene cluster (Fig 3). *isoABCDEFGHIJ, aldH1* and *garB*, encoded polypeptides with high amino acid identity (99.7-100%) to those from *Variovorax* sp. WS11. Indeed, ANI analysis (>99.9%) revealed that *Variovorax* sp. OPL2.2 is the same species as *Variovorax* sp. WS11.

Conclusions

The area of agricultural land dedicated to cultivating oil palm has increased dramatically over the last forty years and continues to increase annually due to palm oil demand from the food industry, domestic products and biofuel production [42].

Growth of a high isoprene-emitting crop and the consequent reactions of isoprene with NOx have raised concerns about the impact of large oil palm plantations on air quality and the regional delivery of the UN sustainable development goals. Therefore, to better understand the role that microbes play in mitigating the effects of this climate-active gas, it is essential to examine the microbial diversity and abundance of isoprene-degrading bacteria. In this study, we show that oil palm harbours a unique and distinct community of isoprene degraders compared to other high isoprene-emitting trees from temperate environments and contains higher numbers of bacteria with the genetic potential to metabolise this climate-active gas, especially soils beneath oil palm trees. Isoprene-utilising bacteria from oil palm soils were phylogenetically more diverse than those from the oil palm phyllosphere, with *Novosphingobium, Pelomonas, Rhodoblastus*, *Sphingomonas* being found in soils and *Gordonia* and *Zoogloea* on oil palm leaves. Analysis of MAGs revealed that bins from these genera contained isoprene degradation gene clusters and amplicon sequencing data showed that oil palm trees contain novel *isoA* sequences, many of which were highly similar to *isoA* genes recovered from *Novosphingobium, Gordonia* or *Rhizobiales* MAGs. The discovery of novel isoprene-degrading bacteria enhances a more robust *isoA* database necessary to utilise the *isoA* probes and assess the distribution, abundance and activity of the isoprene degraders in the environment.

Methods

DNA-stable isotope probing

Three trees from different locations in an oil palm plantation in Palong (Negeri Sembilan, Malaysia) were sampled on 12th November 2018 (Table S6). Soil (50 g)

was collected at a depth of 0-5 cm within the palm circle of three 28-year old oil palm trees after removing vegetation from the surface of the soil. Five healthy leaflets from lower canopy fronds of the corresponding trees were sampled to allow comparison of the diversity of isoprene-degrading bacteria between the oil palm phyllosphere and adjacent soils. Soils and leaf samples were sent to the laboratory of Prof. Niall McNamara at the UK Centre for Ecology and Hydrology in Lancaster, where appropriate facilities to import soils into the UK were available, for processing. Cells were extracted from the soils as follows: 5 g of soils were resuspended in 50 ml sterile distilled water in a sterile 250 ml conical flask. Flasks were then shaken at 150 rpm for 30 min at room temperature to dislodge cells from soil particles. Soil suspensions were decanted into a 50 ml measuring cylinder and left undisturbed for 1 h to allow sedimentation of soil particles. The aqueous layer was decanted into a sterile flask. This treatment was repeated including a sonication step for 5 min in a water bath (Mettler Electronics) to optimise recovery of cells that were attached to soil particles. Soils particles were appropriately disposed of by autoclaving and the aqueous layers of both treatments were then combined and transported in sealed vials to University of East Anglia, where microcosms with soil washings were set up.

Soil DNA-SIP enrichments were set up in triplicate and consisted of 80 ml of combined soil washings in 2 litre air-tight bottles containing 25 ppmv of either ^{12}C or ^{13}C-labelled isoprene. Soil microcosms were incubated at 30 ˚C in the dark with agitation at 150 rpm. Five leaves per oil palm tree (the same trees used for the soil microcosms) were cut into approximately 10 cm length and 5 cm width and placed in sterile glass bottles containing 250 ml sterile distilled water. Leaf samples were sonicated for 5 min in a water bath (Mettler Electronics) and agitated at 150 rpm at room temperature for 1 h to dislodge microbial cells from plant material. Leaf washings were then filtered

through 0.22 µm cellulose nitrate membrane filters (Pall) to concentrate cells. Filters were washed with 40 ml of Ewers minimal medium [62]. Subsequently, filters were discarded, and the washings transferred to 2 litre air-tight bottles to form the basis of the leaf microcosms. Phyllosphere microcosms were then amended with 25 ppmv of either ^{12}C or ^{13}C-labelled isoprene. Leaf DNA-SIP enrichments were set up in triplicate and incubated at 30 ˚C in the dark with shaking (150 rpm). Consumption of isoprene by soil and leaf microcosms was monitored with a Fast Isoprene Sensor (Hills-Scientific) and replenished when the headspace concentration fell below 10 ppmv.

Sampling of soil and leaf DNA-SIP incubations was performed as follows: 10 ml aliquots were collected at T0 and after 5 days (12.5 µmol C assimilated $\cdot g^{-1}$) of incubation for soil microcosms. 10 ml aliquots from microcosms set up with leaf washings were collected at T0 and after 10 days (50 µmol C assimilated $\cdot g^{-1}$) of incubation. Soil and leaf aliquots were then spun down and supernatants were discarded. After that, pellets were resuspended with a 1 ml solution containing sodium phosphate and MT buffers included in the FastDNA Spin Kit for Soil (MP Biomedicals) and transferred to Lysing matrix E tubes to proceed with the DNA extraction according to the manufacturer's instructions. 0.5-2 µg DNA per sample was separated into heavy (^{13}C-labelled) and light (^{12}C-unlabelled) DNA by isopycnic ultracentrifugation as previously described [36]. DNA in each fraction was quantified using a Qubit dsDNA HS Assay kit (ThermoFisher Scientific) following the manufacturer's instructions. The density of each fraction was estimated by refractometry using a Reichert AR200 refractometer (Reichert Analytical Instruments). Heavy and light DNA fractions from each sample were identified by plotting DNA abundance vs fraction density (Fig S5) and used for subsequent downstream analysis.

16S rRNA gene amplicon sequencing

To investigate the bacterial diversity in samples from DNA-SIP experiments, 16S rRNA genes of DNA extracted from unenriched (T0), unfractionated (UF), labelled (heavy; H) and unlabelled (light; L) soil and leaf fractions were amplified with bacterial primers 341F and 785R [63]. Duplicate PCRs for each sample were pooled before purification of PCR amplicons with a High Pure PCR product purification kit (Roche) according to the manufacturer's instructions. It was not possible to obtain amplicons from ^{12}C-heavy DNA (S 12C H) samples arising from SIP experiments with soils due to the low amount of DNA present in these fractions (below the detection limit of 0.2 ng of the Qubit dsDNA HS Assay kit). DNA libraries from purified 16S rRNA gene amplicons were prepared and sequenced at MrDNA (Shallowater, TX, USA) with Illumina MiSeq technology, obtaining an average of 100,757 16S rRNA genes per sample with an average length of 300 bp. Sequence data were processed using MrDNA analysis pipeline. Briefly, reads were first joined and depleted of barcodes. Then, short sequences (<150 bp) and sequences with ambiguous base calls were removed. Resultant sequences were denoised and operational taxonomic units (OTUs) were defined with clustering at 97% similarity, followed by removal of singleton sequences and chimeras. Taxonomy of OTUs was then assigned using BLASTn (http://blast.ncbi.nlm.nih.gov) against a curated database derived from RPDII (http://rdp/cme.msu.edu) and NCBI (www.ncbi.nlm.nih.gov).

isoA gene amplicon sequencing

isoA genes of DNA extracted from ^{13}C-heavy soil and leaf fractions were amplified with primers isoA14F and isoA511R [35]. Duplicate PCRs for each replicate enriched with

490 ^{13}C-isoprene were pooled before purification of PCR amplicons with a High Pure PCR
491 product purification kit (Roche) as above. DNA libraries from purified PCR products
492 were prepared and sequenced at MrDNA (Shallowater, TX, USA) using Illumina MiSeq
493 technology, obtaining an average of 13,542 reads per sample with an average length
494 of 300 bp.

495 *isoA* amplicon sequencing data were analysed with the Bioconductor package DADA2
496 (version 1.6) [64] after demultiplexing the reads and removing primer sequences.
497 Reads were then trimmed to 275 nucleotides and quality-filtered if their expected error
498 was greater than two. Sequences were then denoised using the estimated error rates
499 and resultant reads were dereplicated. Subsequently, forward and reverse reads were
500 merged, chimeric sequences were discarded and the DADA2 algorithm was used to
501 infer individual amplicon sequence variants (ASVs). ASVs were then manually
502 checked by BLASTx [65]. Those ASVs with a top hit distinct from a ratified IsoA
503 sequence were discarded, obtaining a final set of 28 ASVs for soil and 6 ASVs for leaf
504 enrichments.

505

506 Metagenomic analysis of oil palm soil and leaf DNA-SIP samples

507 The 16S rRNA gene profile of each biological replicate of unenriched (T0), labelled
508 (heavy; H) and unlabelled (light; L) fractions from leaf and soil samples was analysed
509 by denaturing gradient gel electrophoresis (DGGE, see below). As they showed highly
510 similar profiles, biological replicates from both unenriched soils (S T0 R1, S T0 R2 and
511 S T0 R3) and phyllosphere (L T0 R1, L T0 R2 and L T0 R3) samples and ^{13}C-heavy
512 fractions from leaves (L 13C H R1, L 13C H R2 and L 13C H R3) were combined in
513 equal proportions for metagenomics sequencing (Fig S6), resulting in samples S T0,

514 L T0 and L 13C H R1-3, respectively. However, the community profile of ^{13}C-heavy
515 fraction from soils replicate 3 (S 13C H R3) showed some differences compared to
516 those from ^{13}C-heavy fractions from replicates 1 and 2 (S 13C H R1 and S 13C H R2;
517 Fig S6). Therefore, ^{13}C-heavy DNA soil fractions from replicates 1 and 2 were pooled
518 (S 13C H R1-2) for downstream analysis, whereas ^{13}C-heavy DNA from replicate 3 (S
519 13C H R3) was sequenced separately. Libraries were prepared by MrDNA
520 (Shallowater, TX, USA), resulting in an average library size of 700 bp for soils T0 (S
521 T0), 710 bp for leaves T0 (L T0), 643 bp for soils ^{13}C-heavy fractions from replicates
522 1 and 2 (S 13C H R1-2), 622 bp for soils ^{13}C-heavy fraction replicate 3 (S 13C H R3)
523 and 639 bp for ^{13}C-heavy leaves fractions (L 13C H R1-3). Libraries were then pooled
524 in equimolar ratios of 1 nM and sequenced as paired ends using the Illumina NovaSeq
525 6000 system.

526 Metagenomic reads were quality-filtered using the iu-filter-quality-minoche script [66]
527 included in Illumina-utils (version 1.4.4) [67], obtaining an average of 19,000,000
528 quality-filtered reads per sample with an average length of 143 bp. Taxonomy of
529 unassembled metagenomes was analysed using MetaPhlAn2 (version 2.0) [49].

530 The abundance of *isoA* genes in unassembled oil palm (this study), willow [32] and
531 poplar [38] unenriched metagenomes was determined by tBLASTn of IsoA sequences
532 from ratified isoprene-degrading bacteria against the raw reads (E ≤ 1e−4). Each
533 potential IsoA sequence retrieved from the analysis of metagenomes was manually
534 checked by BLASTx against a database of IsoA proteins from *bona fide* isoprene
535 degraders and discarded if they showed <50% amino acid identity. Only unique hits
536 were counted. Hit numbers were normalized against read number of the smallest
537 metagenome, to the smallest gene length and to hits of *recA*. Hits of *recA* in
538 unenriched metagenomes were determined by tBLASTn (E ≤ 1e-6) of RecA

sequences from a database obtained from RDP's FunGene [68]. Quality-filtered reads from soil and leaf samples were assembled using metaSPAdes (version 3.13) [69] with kmers 21, 33 and 55, and the quality of each assembly was analysed with MetaQUAST (version 4.6.3) [70]. N50 values were ~1 kb for all metagenome assemblies except for the unenriched soil (S T0) sample, which had an N50 value of 684. Complete statistics of metagenomes assemblies are shown in Table S2.

Assembled contigs were used to reconstruct metagenome assembled genomes (MAGs) using MaxBin2 (version 2.2.2) [59]. MAGs completeness and contamination was assessed and taxonomically assigned using CheckM (version 1.0.18) [60]. A total of 20 MAGs from soil and 52 from leaf samples were obtained. Those MAGs with >75% completeness and <5% contamination were then reassembled and taxonomically verified using the "reassemble_bins" and "classify_bins" modules of the metaWRAP pipeline (version 1.2.2) [71] to improve assembly and increase the likelihood of obtaining full isoprene degradation gene clusters. Local BLAST databases were constructed and screened for the presence of homologues to known isoprene degradation proteins IsoABCDEFGHIJ and other polypeptides associated with the pathway such as AldH1, GshB and GarB using a cut-off value of E<1e-10 in permissive searches, and E<1e-40 in restrictive searches. A total of two MAGs from soils and three from leaves were identified to be of interest based on a completion of >75%, contamination <10% and the presence of IsoABCDEF homologues (Table S3). Finally, MAGs were annotated using Prokka (version 1.13.3) [61].

563 Isolation and characterisation of isoprene-degrading bacteria

564 Once soils and leaf microcosms set up for DNA-SIP experiments had assimilated 50
565 µmol C per g of sample (10 days for leaves and 11 days for soils), they were diluted
566 1/10 into sealed 120 ml serum vials containing 10 ml Ewers minimal medium [62] to
567 isolate isoprene-degrading strains from these environments. Vials were supplemented
568 with 25 ppmv isoprene and incubated at 30 ˚C with shaking (150 rpm). Vials were
569 subcultured three times at 2-week intervals before plating onto Ewers medium agar.
570 These plates were incubated in air-tight jars containing isoprene vapour (1%, v/v) as
571 sole carbon source. After four days, colonies with different morphologies were
572 inoculated in 120 ml serum vials containing 10 ml Ewers medium and 25 ppmv
573 isoprene to determine isoprene consumption of isolates in liquid medium.
574 Consumption of isoprene was monitored using a gas chromatograph fitted with a flame
575 ionization detector as previously described [33]. Those cultures that consistently
576 consumed isoprene were checked for purity by plating onto rich medium R2A agar
577 (Oxoid) and by phase contrast microscopy (Zeiss Axioscop).

578 Growth curves of selected isolates on isoprene were performed as follows: bacterial
579 strains were first grown on Ewers medium with glucose (10 mM) for 48 h at 30 °C.
580 Cultures were then pelleted and washed three times with Ewers medium with no
581 carbon sources and adjusted to an OD_{600} of 0.8. Cell suspensions were then
582 inoculated into 120 ml serum vials containing 20 ml of fresh Ewers medium amended
583 with isoprene vapour (5%, v/v) as sole carbon source. Cultures were incubated at 30
584 °C and growth was estimated by measuring cell density at OD_{600} with a UV-1800
585 spectrophotometer (Shimadzu).

Genomic DNA of isoprene-degrading strains was extracted using a Wizard Genomic DNA purification kit (Promega) according to the manufacturer's guidelines. The phylogeny of isolates was identified by PCR amplification of their 16S rRNA genes with primers 27F and 1492R [72] and subsequent Sanger sequencing (Eurofins genomics). Genomic DNA was also examined for the presence of *isoA* genes using PCR primers isoA14F and isoA511R, which are specific for the detection of *isoA*, encoding the IsoMO α-subunit [35]. PCR conditions for amplification of *isoA* genes consisted of an initial step of 94 °C for 2 min, followed by 31 cycles of 95 °C for 15 s, 54 °C for 30 s, 72 °C for 1 min and a final extension step of 72 °C for 7 min as described in [35].

Illumina and Nanopore sequencing of genomic DNA from *Variovorax* sp. OPL2.2

Genomic DNA from *Variovorax* sp. OPL2.2 for Illumina and Nanopore sequencing was extracted using a phenol: chloroform: isoamyl alcohol method described by Wilson [73]. For Illumina sequencing, library preparation was done using the Nextera XT kit (Illumina) according to the manufacturer's instructions. The library was checked for quality and size distribution using the Agilent 2100 Bioanalyzer (Agilent) and the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). The library was then denatured and sequenced with Illumina Miseq technology (San Diego, California USA). Paired-end sequencing of 2 x 300 bp was performed using the MiSeq Reagent Kit v3 (San Diego, California USA) according the manufacturer's protocol. Illumina sequencing of *Variovorax* sp. OPL2.2 genomic DNA resulted in 1,274,620 reads.

Genomic DNA (0.9 µg) from *Variovorax* sp. OPL2.2 was used to prepare the library for Nanopore sequencing. Library construction was performed using the Ligation

610 Sequencing Kit 1D (SQK-LSK109) in combination with the Native barcoding
611 Expansion Kit (EXP-NBD104) according the manufacturer's protocol (Oxford
612 Nanopore Technologies). DNA fragments were repaired and A-tailed using the
613 NEBNext® FFPE DNA Repair Mix and NEBNext® Ultra™ II End Repair/dA-Tailing
614 Module (New England Biolabs). After purification with AMPure XP beads (Beckman
615 Coulter Life Sciences), selected barcodes were ligated using the Blunt/TA Ligase
616 Master Mix (New England Biolabs). After ligation of the barcodes and bead clean-up,
617 the library was quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher
618 Scientific). Thereafter, adapters were ligated using the NEBNext® Quick Ligation
619 Module (New England Biolabs). The library was then purified and quantified with the
620 Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and sequenced using a Flow
621 Cell (R9.4.1) and MinION device (Oxford Nanopore Technologies) according to the
622 manufacturer's instructions.

623 Nanopore sequencing resulted in 44,530 reads (N50 = 10.5 kb). Base-calling and de-
624 multiplexing after sequencing was done using the Guppy Base-calling Software
625 (Oxford Nanopore Technologies, Limited Version 3.2.4+d9ed22f) resulting in 13,966
626 reads, selecting for a minimal sequence length of 3,000 bp.

627 Assembly of both Illumina and Nanopore reads to obtain a full-length genome was
628 done using Unicycler [74]. Assembly of *Variovorax* sp. OPL2.2 reads yielded 50
629 contigs with a total length of 8,516,444 bp, and the largest contig being 2,344,322 bp.
630 Completeness of the assembled genome was analysed by CheckM [60] resulting in
631 98.4% completeness and 1.1% contamination. Automatic annotation of *Variovorax* sp.
632 OPL2.2 genome was performed using Prokka [61].

633 Finally, average nucleotide identity (ANI) between *Variovorax* sp. OPL2.2 and
634 *Variovorax* sp. WS11 [32, 38] was calculated using the online ANI calculator available
635 on the Kostas Lab website (http://enve-omics.ce.gatech.edu/ani/index, accessed
636 10/12/2019).

637

638 Denaturing gel gradient electrophoresis

639 Bacterial 16S rRNA genes from DNA samples arising from DNA-SIP experiments were
640 amplified using primers 341F-GC [75] and 907R [76]. To visualise 16S rRNA gene
641 profiles of the bacterial communities from soil and leaf incubations, denaturing gel
642 electrophoresis (DGGE) was performed following the protocol described by El
643 Khawand *et al.* [36].

644

645 **Additional files**

646 **Additional file 1: Fig S1.** Bacterial diversity profile of the oil palm unenriched (T0)
647 community at the phyla level. **Fig S2.** Bacterial community profiles of oil palm soil
648 samples analysed by 16S rRNA gene amplicon sequencing. **Fig S3.** Bacterial
649 community composition of oil palm leaf samples analysed by 16S rRNA gene amplicon
650 sequencing. **Fig S4.** Growth curve of *Variovorax* sp. OPL2.2 on isoprene as sole
651 carbon and energy source. **Fig S5.** DNA retrieved as function of density of each
652 fraction recovered after isopycnic ultracentrifugation. **Fig S6.** 16S rRNA gene profiles
653 of oil palm soil and phyllosphere samples analysed by DGGE. **Table S1.** Relative
654 abundance of key isoprene-degrading bacterial genera in oil palm soil and leaf
655 samples. **Table S2.** Statistics for metagenome assemblies. **Table S3.** Metagenome
656 assembled genomes (MAGs) that contain genes encoding proteins homologous to

IsoABCDEF (E<1e-40). **Table S4.** MAGs genes encoding polypeptides homologous to proteins involved in isoprene metabolism from ratified isoprene-degrading strains. **Table S5.** ASVs retrieved from *isoA* amplicon sequencing analysis of ^{13}C-heavy DNA from soil and leaf incubations. **Table S6.** Location of oil palm trees used to set up soil and leaf DNA-SIP incubations (docx 2.0 Mb).

Acknowledgements

We thank Andrew Crombie for insightful comments on the manuscript and Robin Dawson, Nasmille Larke-Mejía, Colin Lockwood, and Leanne Sims for useful scientific discussions. We acknowledge field and laboratory support from Nur Izzati Ridzuan, University of Nottingham, Malaysia and thank Professor Ir. And Dr. Denny K. S. Ng from Herriot Watt University, Malaysia, for assisting with access to plantations. Bioinformatics analyses in this study were conducted in the High-Performance Computing Cluster supported by the Research and Specialist Computing Support Service at the University of East Anglia, UK.

Funding

The work on this project was funded through a European Research Council Advanced grant to JCM (694578—IsoMet), a Natural Environment Research Council grant (NE/J009725/1) and the Earth and Life Systems Alliance (ELSA) at the University of East Anglia. Work in HJMOdC laboratory was supported by the European Research Council (ERC Advanced Grant project VOLCANO 669371). TJM was funded by a Natural Environment Research Council grant (NE/J009555/1). Work in NPMcN

laboratory was funded by the UK Natural Environment Research Council (SUNRISE project; NE/R000131/1).

Availability of data and materials

Amplicon sequencing and metagenomic data generated in this study were deposited to the sequence read archives (SRA) under Bioproject PRJNA272922 (Biosamples SAMN14771267 - SAMN14771280).

Authors' contributions

OCF, LG, TJM and JCM planned the experiments. OCF and LG carried out the experimental work and analysed results. LG designed and performed bioinformatics analyses. DMOE, NPMcN and CVS collected oil palm soil and phyllosphere samples in Malaysia. TAvA and HJMOdC performed the Illumina and Nanopore sequencing of genomic DNA from *Variovorax* sp. OPL2.2. OCF, LG and JCM wrote the manuscript with contributions from all authors. All authors read and approved the manuscript before submission.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

1. Atkinson R, Arey J. Gas-phase tropospheric chemistry of biogenic volatile organic compounds: A review. Atmos Environ. 2003;37:197-219.
2. Guenther AB, Jiang X, Heald CL, Sakulyanontvittaya T, Duhl T, Emmons LK, *et al*. The model of emissions of gases and aerosols from nature version 2.1 (MEGAN2.1): An extended and updated framework for modeling biogenic emissions. Geosci Model Dev. 2012;5:1471–92.
3. United Nations Statistics Division: The sustainable development goals report 2019. https://unstats.un.org/sdgs/report/2019 (2019). Accessed 6 Mar 2020.
4. Griggs D, Stafford-Smith M, Gaffney O, Rockström J, Öhman MC, Shyamsundar P, *et al*. Sustainable development goals for people and planet. Nature. 2013;495:305-7.
5. Monson RK, Trahan N, Rosenstiel TN, Veres P, Moore D, Wilkinson M, *et al*. Isoprene emission from terrestrial ecosystems in response to global change: minding the gap between models and observations. Philos Trans A Math Phys Eng Sci. 2007;365:1677-95.
6. Sanderson MG, Jones CD, Collins WJ, Johnson CE, Derwent RG. Effect on climate change on isoprene emissions and surface ozone levels. Geophys Res Lett. 2003;30:1936.
7. Engelhart GJ, Moore RH, Nenes A, Pandis SN. Cloud condensation nuclei activity of isoprene secondary organic aerosol. J Geophys Res. 2011;116:D02207.
8. Morais ARC, Dworakowska S, Reis A, Gouveia L, Matos CT, Bogdal D, *et al*. Chemical and biological-based isoprene production: Green metrics. Catal Today. 2015;239:38-43.
9. Dani SKG, Benavides AMS, Michelozzi M, Peluso G, Torzillo G, Loreto F. Relationship between isoprene emission and photosynthesis in diatoms, and its implications for global marine isoprene estimates. Mar Chem. 2017;189:17–24.
10. Geron C, Owen S, Guenther A, Greenberg A, Rasmussen R, Hui Ban J, *et al*. Volatile organic compounds from vegetation in southern Yunnan Province, China: Emission rates and some potential regional implications. Atmos Environ. 2006;40:1759-73.

11. Bäck J, Aaltonen H, Hellén H, Kajos MK, Patotoski J, Taipale R, *et al*. Variable emissions of microbial volatile organic compounds (MVOCs) from root-associated fungi isolated from Scots pine. Atmos Environ. 2010;44:3651-9.
12. Exton DA, Suggett DJ, McGenity TJ, Steinke, M. Chlorophyll-normalized isoprene production in laboratory cultures of marine microalgae and implications for global models. Limnol Oceanogr. 2013;58:1301–11.
13. Fall R, Copley SD. Bacterial sources and sinks of isoprene, a reactive atmospheric hydrocarbon. Environ Microbiol. 2000;2:123–130.
14. Kuzma J, Nemecek-Marshall M, Pollock WH, Fall R. Bacteria produce the volatile hydrocarbon isoprene. Curr Microbiol. 1995;30:97–103.
15. Shaw SL, Gantt B, Meskhidze N. Production and emissions of marine isoprene and monoterpenes: a review. Adv Meterol. 2010:408696.
16. Steinke M, Hodapp B, Subhan R, Bell TG, Martin-Creuzburg D. Flux of the biogenic volatiles isoprene and dimethyl sulphide from an oligotrophic lake. Sci Rep. 2018;8:630.
17. Logan BA, Monson RK, Potosnak MJ. Biochemistry and physiology of foliar isoprene production. Trends Plant Sci. 2000;5:477–81.
18. Loreto F, Ciccioli P, Brancaleoni E, Valentini R, De Lillis M, Csiky O *et al*. A hypothesis on the evolution of isoprenoid emission by oaks based on the correlation between emission type and *Quercus* taxonomoy. Oeacologia. 1998;115:17430-35.
19. Monson RK, Jones RT, Rosenstiel TN, Schnitzler JP. Why only some plants emit isoprene. Plant Cell Environ. 2013;36:503-16.
20. Sharkey TD. Is it useful to ask why plants emit isoprene? Plant Cell Environ. 2013;36:517-20.
21. Rohmer, M. The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. Nat Prod Rep. 1999;16:565-74.
22. Monson RK, Fall R. Isoprene emission from aspen leaves: influence of environment and relation to photosynthesis and photorespiration. Plant Physiol. 1989;90:267-74.
23. Sharkey TD, Yeh S. Isoprene emission from plants. Annu Rev Plant Physiol Plant Mol Biol. 2001;52:407-36.
24. Behnke K, Ehlting B, Teuber M, Bauerfeind M, Louis S, Hänsch R *et al*. Transgenic, non-isoprene emitting plants don't like it hot. Plant J. 2007;51:485-99.

25. Laothawornkitkul J, Paul ND, Vickers CE, Possell M, Taylor JE, Mullineaux PM *et al*. Isoprene emissions influence herbivore feeding decisions. Plant Cell Environ. 2008;31:1410-5.
26. Vickers CE, Possell M, Cojocariu CI, Velikova VB, Laothawornkitkul J, Ryan A *et al*. Isoprene synthesis protects transgenic tobacco plants from oxidative stress. Plant Cell Environ. 2009;32-520-31.
27. Lantz AT, Allman J, Weraduwage SM, Sharkey TD. Isoprene: New insights into the control of emission and mediation of stress tolerance by gene expression. Plant Cell Environ. 2019;42:2808-26.
28. Cleveland CC, Yavitt JB. Consumption of atmospheric isoprene in soil. Geophys Res Lett. 1997;24-2379-82.
29. Cleveland CC, Yavitt JB. Microbial consumption of atmospheric isoprene in a temperate forest soil. Appl Env Microbiol. 1998;64:172–77.
30. Gray CM, Helmig D, Fierer N. Bacteria and fungi associated with isoprene consumption in soil. Elem Sci Anth. 2015;3:000053.
31. McGenity TJ, Crombie AT, Murrell JC. Microbial cycling of isoprene, the most abundantly produced biological volatile organic compound on Earth. ISME J. 2018;12:931-41.
32. Larke-Mejía NL, Crombie AT, Pratscher J, McGenity TJ, Murrell JC. Novel isoprene-degrading proteobacteria from soil and leaves identified by cultivation and metagenomics analysis of stable isotope probing experiments. Front Microbiol. 2019;10:2700.
33. Crombie AT, Khawand ME, Rhodius VA, Fengler KA, Miller MC, Whited GM *et al.* Regulation of plasmid-encoded isoprene metabolism in *Rhodococcus*, a representative of an important link in the global isoprene cycle. Environ Microbiol. 2015;17:3314-29.
34. Leahy JG, Batchelor PJ, Morcomb SM. Evolution of the soluble diiron monooxygenases. FEMS Microbiol Rev. 2003;27:449-479.
35. Carrión O, Larke-Mejía NL, Gibson L, Farhan Ul Haque M, Ramiro-García J, McGenity TJ, *et al*. Gene probing reveals the widespread distribution, diversity and abundance of isoprene-degrading bacteria in the environment. Microbiome. 2018;6:219.
36. El Khawand M, Crombie AT, Johnston A, Vavlline DV, McAuliffe JC, Latone JA *et al.* Isolation of isoprene degrading bacteria from soils, development of *iso*A gene probes and identification

of the active isoprene-degrading soil community using DNA-stable isotope probing. Environ Microbiol; 2016;18:2743–53.

37. Neufeld JD, Vohra J, Dumont MG, Lueders T, Manefield M, Friedrich MW, *et al.* DNA stable-isotope probing. Nat Protoc. 2007;2:860–6.
38. Crombie AT, Larke-Mejía NL, Emery H, Dawson R, Pratscher J, Murphy GP, *et al.* Poplar phyllosphere harbors disparate isoprene-degrading bacteria. Proc Natl Acad Sci USA. 2018;115:13081-6.
39. Kesselmeier J, Staudt M. Biogenic volatile organic compounds (VOC): An overview on emission, physiology and ecology. J Atmos Chem. 1999;33:23-88.
40. Cheng Y, Yu L, Xu Y, Lu H, Cracknell AP, Kanniah K *et al*. Mapping oil palm plantation expansion in Malaysia over the past decade (2007-2016) using ALOS-1/2 PALSAR-1/2 data. Int J Remote Sens. 2019;40:7389-408.
41. Ibragimov A, Sidique SF, Tey YS. Productivity for sustainable growth in Malaysian oil palm production: a system dynamics modeling approach. J Clean Prod. 2019;213:1051–62.
42. Hewitt CN, MacKenzie AR, Di Carlo P, Di Marco CF, Dorsey JR, Evans M *et al*. Nitrogen management is essential to prevent tropical oil palm plantations from causing ground-level ozone pollution. Proc Natl Acad Sci USA. 2009;106:18447-51.
43. Fulthorpe RR, Roesch LF, Riva A, Triplett EW. Distantly sampled soils carry few species in common. ISME J. 2008;2:901-10.
44. Janssen PH. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Appl Environ Microbiol. 2006;72:1719-28.
45. Karimi B, Terrat S, Dequiedt S, Saby NPA, Horrigue W, Lelièvre M, *et al*. Biogeography of soil bacteria and archaea across France. Sci Adv. 2018;4:eaat1808.
46. Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AK, Kent AD, *et al*. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J. 2007;1:283-90.
47. Acuña Alvarez LA, Exton DA, Timmis KN, Suggett DJ, McGenity TJ. Characterization of marine isoprene-degrading communities. Environ Microbiol. 2009;11:3280–91.
48. Johnston A, Crombie AT, Khawand ME, Sims L, Whited GM, McGenity TJ *et al.* Identification and characterisation of isoprene-degrading bacteria in an estuarine environment. Environ Microbiol. 2017;19:3526–37.

819 49. Truong DT, Franzosa EA, Tickle TL, Scholz M, Weingart G, Pasolli E, *et al*. MetaPhlAn2 for
820 enhanced metagenomic taxonomic profiling. Nat Methods. 2015;12:902-3.
821 50. Kembel SW, O'Connor TK, Arnold HK, Hubbell SP, Wright SJ, *et al*. Relationships between
822 phyllosphere bacterial communities and plant functional traits in a neotropical forest. Proc Natl
823 Acad Sci USA. 2014;111:13715-20.
824 51. Lambais MR, Crowley DE, Cury JC, Büll RC, Rodrigues RR. Bacterial diversity in tree canopies
825 of the Atlantic forest. Science. 2006;312:1917.
826 52. Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. The ecology of the phyllosphere:
827 geographic and phylogenetic variability in the distribution of bacteria on tree leaves. Environ
828 Microbiol. 2010;12:2885-93.
829 53. Vorholt JA. Microbial life in the phyllosphere. Nat Rev Microbiol. 2012;10:828-40.
830 54. Knief C, Delmotte N, Chaffron S, Stark M, Innerebner G, Wassmann R, von Mering C, Vorholt
831 JA. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere
832 of rice. ISME J. 2012;6:1378-90.
833 55. Rastogi G, Sbodio A, Tech JJ, Suslow TV, Coaker GL, Leveau JHJ. Leaf microbiota in an
834 agroecosystem: spatiotemporal variation in bacterial community composition on field-grown
835 lettuce. ISME J. 2012;6:1812-22.
836 56. Bodenhausen N, Horton MW, Bergelson J. Bacterial communities associated with the leaves
837 and the roots of *Arabidopsis thaliana*. PLoS One. 2013;8:e56329.
838 57. Wei YJ, Wu Y, Yan YZ, Zou W, Xue J, Ma WR, *et al*. High-throughput sequencing of microbial
839 community diversity in soil, grapes, leaves, grape juice and wine of grapevine from China. PLoS
840 One. 2018;13:e0193097.
841 58. Rasmussen RA, Khalil MAK. Isoprene over the Amazon basin. J Geophys Res-Atmos.
842 1988;93:1417-21.
843 59. Wu YW, Simmons BA, Singer SW. MaxBin 2.0: an automated binning algorithm to recover
844 genomes from multiple metagenomic datasets. Bioinformatics. 2016;32:605-7.
845 60. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the
846 quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome
847 Res. 2015;25:1043-55.
848 61. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30:2068-9.

62. Dorn E, Hellwig M, Reineke W, Knackmuss HJ. Isolation and characterization of a 3-chlorobenzoate degrading pseudomonad. Arch Microbiol. 1974;99:61–70.
63. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013;41:e1.
64. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581-3.
65. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, *et al*. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:421.
66. Minoche AE, Dohm JC, Himmelbauer H. Evaluation of genomic high-throughput sequencing data generated on Illumina HiSeq and genome analyzer systems. Genome Biol. 2011;12:R112.
67. Eren AM, Vineis JH, Morrison HG, Sogin ML. A filtering method to generate high quality short reads using illumina paired-end technology. PLoS One. 2013;8:e66643.
68. Fish JA, Chai B, Wang Q, Sun Y, Brown CT, Tiedje JM, *et al*. FunGene: the functional gene pipeline and repository. Front Microbiol. 2013;4:291.
69. Nurk S, Meleshko D, Korobeynikov A, Pevzner PA. metaSPAdes: a new versatile metagenomics assembler. Genome Res. 2017;27:824-34.
70. Mikheenko A, Saveliev V, Gurevich A. MetaQUAST: evaluation of metagenome assemblies. Bioinformatics. 2016;32:1088-90.
71. Uritskiy GV, DiRuggiero J, Taylor J. MetaWRAP-a flexible pipeline for genome-resolved metagenomic data analysis. Microbiome. 2018;6:158.
72. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M, editors. Nucleic acid techniques in bacterial systematics. Chichester: John Wiley & Sons; 1991. p. 115-75.
73. Wilson K. Preparation of genomic DNA from bacteria. Curr Protocol Mol Biol. 2001;56:2.4.1-2.45.
74. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017;13:e1005595.
75. Muyzer G, de Waal EC, and Uitterlinden, AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol. 1993;59: 695–700.

76. Muyzer G, Brinkhoff T, Nuebel U, Santegoeds C, Schäefer H, Waver C. Denaturing Gradient Gel Electrophoresis (DGGE) in microbial ecology. In: Akkermans ADL, van Elsas JD, de Bruijim FJ, editors. Molecular microbial ecology manual. Dordrecht: Kluwer Academic Publishers; 1998. p. 1-27.

Figure legends

Fig 1. Bacterial community profile of oil palm soil samples. Bacterial diversity of the unenriched (T0) and labelled (heavy; H) fractions of ^{13}C-isoprene soil incubations was analysed by 16S rRNA gene amplicon (16S) and metagenomics (MG) sequencing. The unenriched bacterial community (S T0) represents the average of three biological replicates. ^{13}C-heavy DNA from replicates 1 and 2 were combined before MG sequencing due to their highly similar 16S rRNA gene community profile shown by DGGE, resulting in sample S 13C H R1-2 (see Methods). Only genera with >5 RA% in at least one of the conditions are represented. Genera present at >10% in any sample are shown in bold. Genera with <5% RA are grouped in “Others”. For complete 16S rRNA gene amplicon sequencing data, including individual replicates and ^{12}C-isoprene controls, see Fig S2.

Fig 2. Bacterial community composition of oil palm phyllosphere samples. Bacterial diversity profile of unenriched (T0) and labelled (heavy; H) fractions of ^{13}C-isoprene incubations of oil palm leaf samples was analysed by 16S rRNA gene amplicon (16S) and metagenomics (MG) sequencing. The unenriched bacterial community (L T0) represents the average of three biological replicates. ^{13}C-heavy DNA from replicates 1, 2 and 3 of leaf incubations were combined before MG sequencing due to their highly similar 16S rRNA gene community profile shown by DGGE, resulting in sample L 13C H R1-3 (see Methods). Only genera with >5 RA% in at least one of the conditions are represented. Genera present at >10% in any sample are shown in bold. Genera with <5% RA are grouped in “Others”. For

complete 16S rRNA gene amplicon sequencing data, including individual replicates and ^{12}C-isoprene controls, see Fig S3.

Fig 3. Isoprene metabolic gene clusters from representative isoprene-degrading strains (in bold) and metagenome assembled genomes (MAGs). Genes encoding IsoMO (*isoABCDEF*) are coloured in red. Adjacent genes not involved in isoprene degradation are coloured in white. Regulatory genes are shown in black. "\\" represents a discontinuity between two DNA contigs. *Variovorax* sp. OPL2.2 was isolated in this study from oil palm leaf enrichments.

Fig 4. Relative abundance and diversity of *isoA* genes in ^{13}C-heavy fractions from oil palm incubations. *isoA* sequences in ^{13}C-heavy fractions from oil palm soil (S 13C H) and leaf (L 13C H) samples were analysed by *isoA* amplicon sequencing (see Methods). Only AVSs with >5% RA in at least one replicate are represented. ASVs present at >10% in any sample are shown in bold. ASVs closely related to IsoA from *Rhizobiales* MAG are represented in purple; ASVs with highest homology to IsoA from *Novosphingobium* MAG are shown in green; ASVs closely related to IsoA from *Rhodococcus* are coloured in blue; ASVs with highest homology to IsoA from *Gordonia* MAG are represented in orange. ASVs with RA <5% are grouped as "Others". For complete *isoA* gene amplicon sequencing data, see Table S5.

Additional File 1

Diversity of isoprene-degrading bacteria in phyllosphere and soil communities from a high isoprene-emitting environment: a Malaysian oil palm plantation

Ornella Carrión[1‡*], Lisa Gibson[1‡], Dafydd M.O. Elias[2], Niall P. McNamara[2], Theo A. van Alen[3], Huub J.M. Op den Camp[3], Christina Vimala Supramaniam[4], Terry J. McGenity[5], J. Colin Murrell[1*]

[1]School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK

[2]Centre of Ecology and Hydrology, Lancaster University, Bailrigg, Lancaster, LA1 4AP, UK

[3]Department of Microbiology, Faculty of Science, IWWR, Radboud University Nijmegen, Heyendaalseweg 135, NL-6525 AJ Nijmegen, The Netherlands

[4]School of Biosciences, Nottingham Centre of Sustainable Palm Oil, University of Nottingham-Malaysia, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia

[5]School of Life Sciences, University of Essex, Colchester, UK

[‡]These authors contributed equally to this work

*Corresponding authors:

J Colin Murrell, [1]School of Environmental Sciences, University of East Anglia

Norwich Research Park, NR4 7TJ, UK

E-mail: j.c.murrell@uea.ac.uk

Tel: (+44) 01603 592959

Ornella Carrión, [1]School of Environmental Sciences, University of East Anglia

Norwich Research Park, NR4 7TJ, UK

E-mail: o.carrion-fonseca@uea.ac.uk

Tel: (+44) 01603 592239

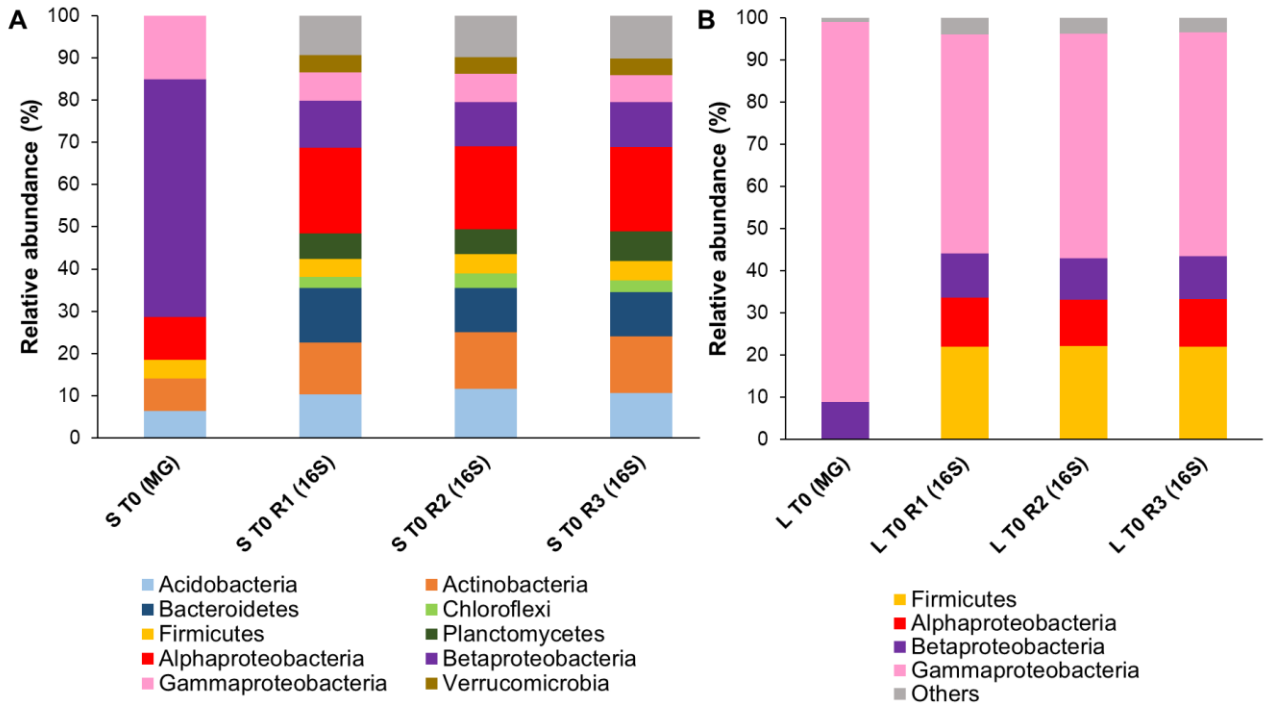


Fig S1. Bacterial diversity profile of the oil palm unenriched (T0) community at the phyla level. Bacterial community composition was analysed by 16S rRNA gene amplicon (16S) and metagenomics (MG) sequencing. A: soil T0 samples; B: phyllosphere T0 samples. Only phyla with >2% relative abundance in at least one of the samples are represented. The Proteobacteria phylum is subdivided into Alpha-, Beta- and Gammaproteobacteria classes. Bacterial phyla with <2% relative abundance are recorded as “Others”.

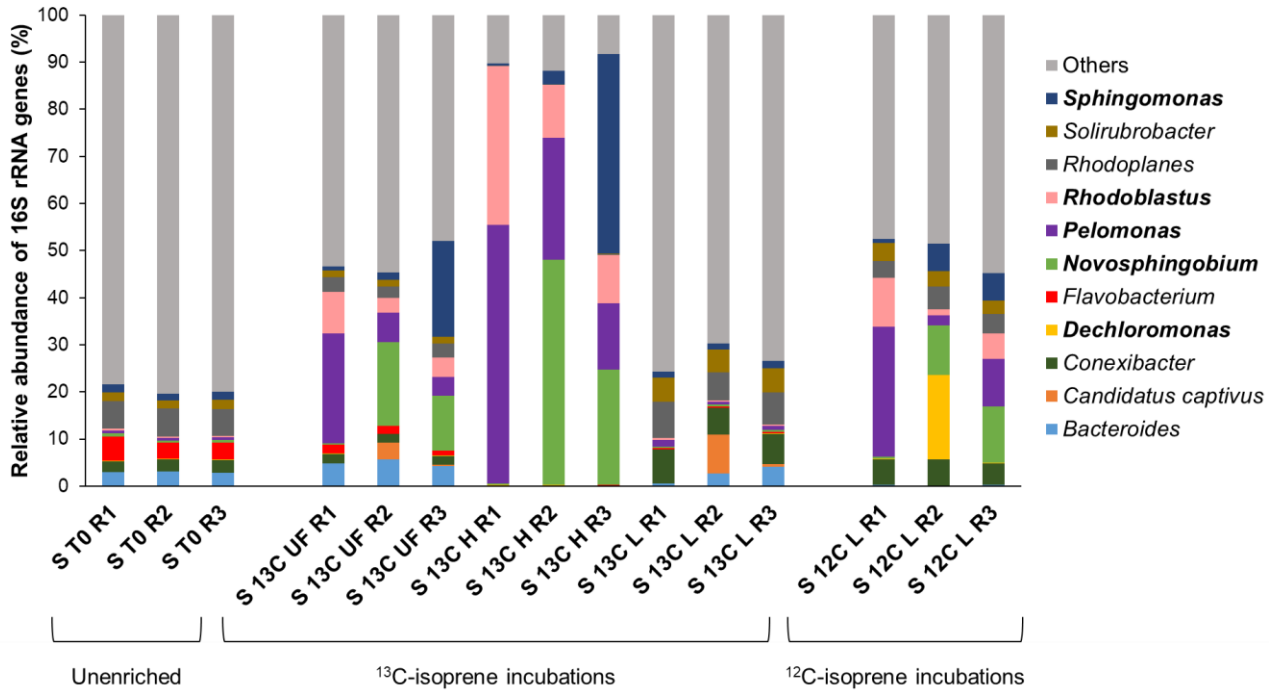


Fig S2. Bacterial community profiles of oil palm soil samples analysed by 16S rRNA gene amplicon sequencing. T0: unenriched samples; 13C UF: unfractionated DNA from ^{13}C-isoprene enrichments; 13C H: heavy DNA from ^{13}C-isoprene incubations; 13C L: light DNA from ^{13}C-isoprene samples; 12C L: light DNA from ^{12}C-isoprene control samples. Only OTUs with >5% relative abundance in at least one of the replicates are represented. Genera present at >10% in any sample are shown in bold. OTUs with <5% relative abundance are shown as “Others”.

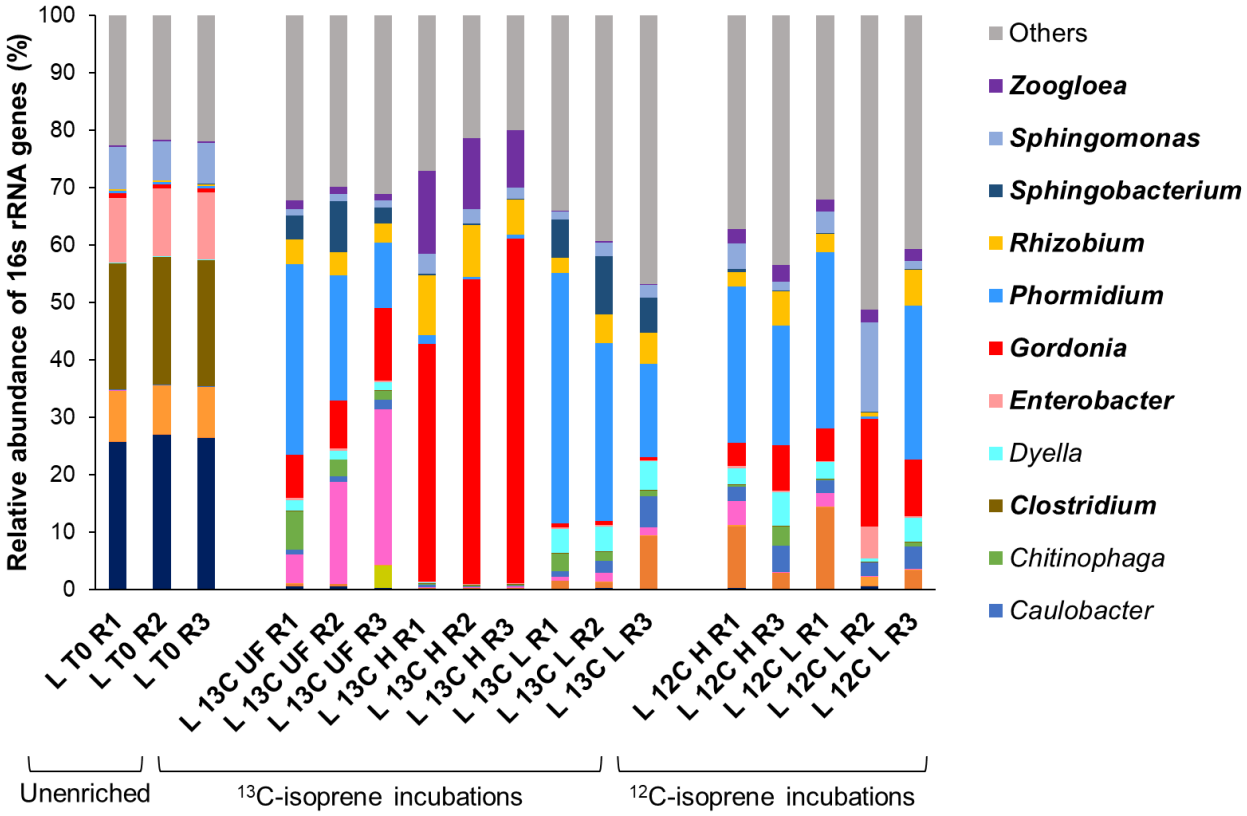


Fig S3. Bacterial community composition of oil palm leaf samples analysed by 16S rRNA gene amplicon sequencing. T0: unenriched samples; 13C UF: unfractionated DNA from ^{13}C-isoprene enrichments; 13C H: heavy DNA from ^{13}C-isoprene incubations; 13C L: light DNA from ^{13}C-isoprene samples; 12C H: heavy DNA from ^{12}C-isoprene control incubations; 12C L: light DNA from ^{12}C-isoprene control samples. Only OTUs with >5% relative abundance in at least one of the replicates are represented. Genera present at >10% in any sample are shown in bold. OTUs with <5% relative abundance are recorded as “Others”.

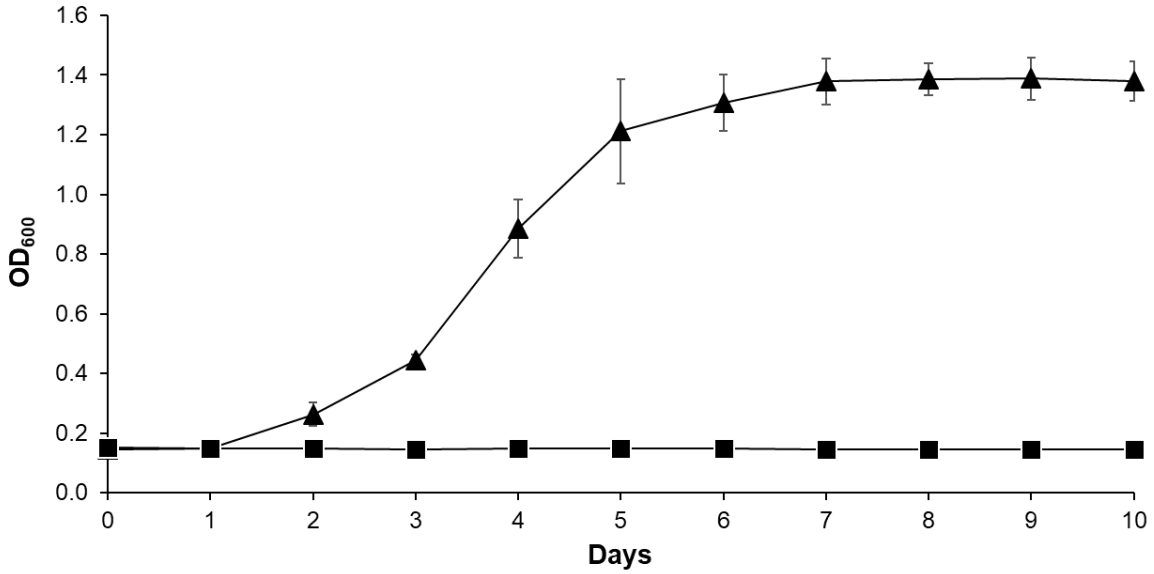


Fig S4. Growth curve of *Variovorax* sp. OPL2.2 on isoprene as sole carbon and energy source. Triangles: *Variovorax* sp. OPL2.2 cultures amended with isoprene (5%; v/v). Data points represent the average of three biological replicates with their respective standard deviations. Squares: *Variovorax* sp. OPL2.2 culture with no carbon source added (control; one replicate).

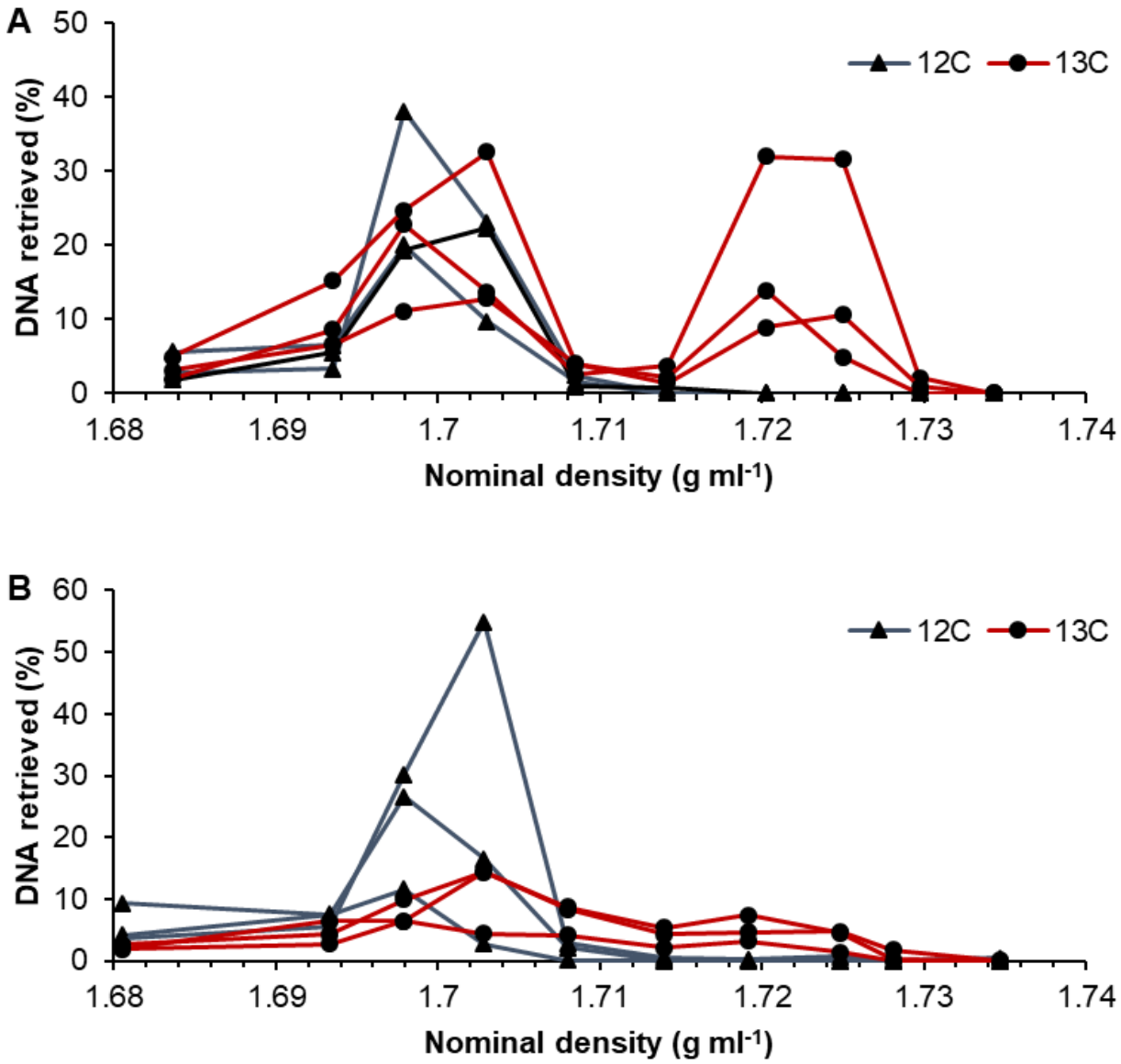


Fig S5. DNA retrieved as function of density of each fraction recovered after isopycnic ultracentrifugation. A: soil samples incubated with ^{12}C (blue line) or ^{13}C-isoprene (red line); B: leaf incubations with ^{12}C (blue line) or ^{13}C-isoprene (red line).

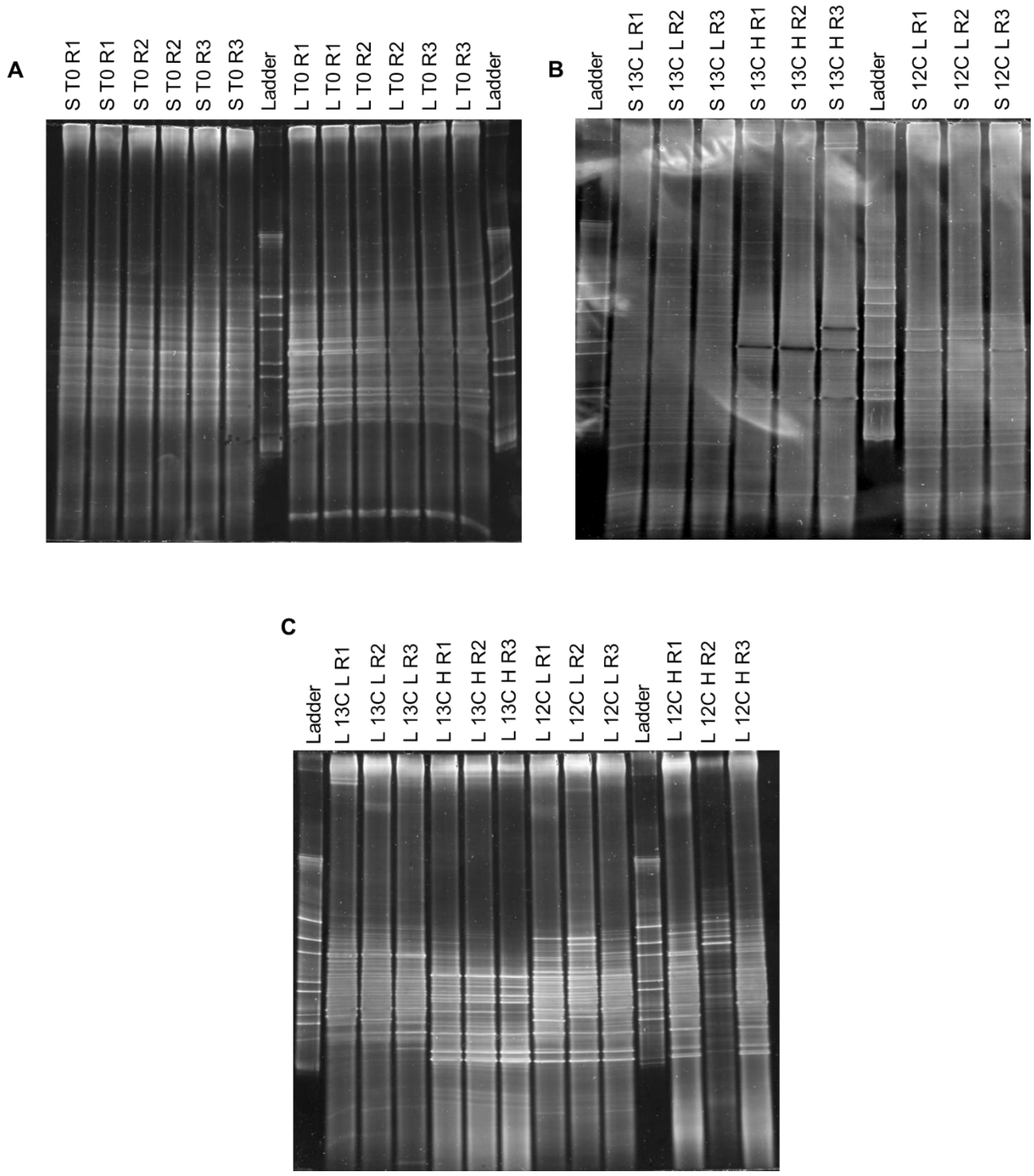


Fig S6. 16S rRNA gene profiles of oil palm soil and phyllosphere samples analysed by DGGE. A: biological replicates from unenriched soil (S T0) and phyllosphere (L T0) samples, each of which was ran in duplicate. B: biological replicates from heavy (H) and light (L) soil incubations with ^{13}C or ^{12}C-isoprene (control). C: biological replicates from heavy (H) and light (L) leaf samples enriched ^{13}C or ^{12}C-isoprene.

Table S1. Relative abundance of key isoprene-degrading bacterial genera in oil palm soil and leaf samples.

Genus	S T0	L T0	S 13C H	L 13C H
Gordonia	0.7 ± 0.2%	0.8 ± 0.1%	0.9 ± 0.1%	51.4 ± 9.3%
Novosphingobium	0.6 ± 0.1%	2.8 ± 0.1%	24.2 ± 23.8%	0.3 ± 0.1%
Pelomonas	0.5 ± <0.1%	0.4 ± 0.1%	31.7 ± 21.0%	0.4 ± 0.1%
Rhodoblastus	0.4 ± 0.1%	0.2 ± <0.1%	18.4 ± 13.3%	0.3 ± 0.1%
Sphingomonas	1.5 ± 0.1%	7.1 ± 0.3%	15.2 ± 23.6%	2.6 ± 0.7%
Zoogloea	0.2 ± <0.1%	0.3 ± 0.1%	0.3 ± 0.1%	12.3 ± 2.2%

Relative abundance of bacterial genera in oil palm incubations was analysed by 16S rRNA gene amplicon sequencing. Values shown represent the average of three biological replicates with their respective standard deviations. S T0: unenriched soil samples; L T0: unenriched leaf samples; S 13C H: heavy DNA from soil samples enriched with ^{13}C-isoprene; L 13C H: heavy DNA from leaf samples enriched with ^{13}C-isoprene.

Table S2. Statistics for metagenome assemblies.

	S T0	S 13C H R1-2	S 13C H R3	L T0	L 13C H R1-3
Contigs	138,886	36,166	22,852	76,131	114,878
Largest contig (bp)	75,971	703,868	1,215,493	732,750	621,426
Total length (bp)	101,440,201	64,831,442	49,671,873	152,876,094	150,393,192
GC (%)	63.9	64.9	64.6	56.5	66
N50	684	2,852	5,677	5,018	1,565
N75	568	1,078	1,322	1,171	798
L50	50,076	3,502	959	3,953	18,953
L75	91,069	13,317	6,174	21,847	54,079
Bins	4	9	7	28	24

Metagenomes from unenriched soil (S T0) and leaf (L T0) samples and ^{13}C-heavy fractions from soil (S 13C H R1-2 and S 13C H R3) and leaf (L 13C H R1-3) incubations were assembled with metaSPAdes using kmers 21, 33 and 55. Quality of the assemblies was analysed by MetaQUAST and bins obtained using MaxBin2.

Table S3. Metagenome assembled genomes (MAGs) that contain genes encoding proteins homologous to IsoABCDEF (E<1e-40).

MAG	Metagenome of origin	Size (Mbp)	N50	Completeness (%)	Contamination (%)	Strain heterogeneity (%)
Novosphingobium	S 13C H R3	3.7	441,005	99.5	<0.1	0
Rhizobiales	S 13C H R3	4.1	59,990	97.6	2.5	79
Gordonia polyisoprenovorans	L 13C H R1-3	6.1	194,257	99.8	0.9	40
Zoogloeaceae	L 13C H R1-3	5.2	49,423	98.7	2.1	10
Ralstonia	L 13C H R1-3	4.5	5,039	79	6.4	0

MAGs were reconstructed from metagenomic sequencing of ^{13}C-heavy DNA from soil (S 13C H) and leaf (L 13C H) incubations with ^{13}C-isoprene. MAGs completeness and contamination was assessed and taxonomically assigned using CheckM (see Methods). N50 is calculated for contigs.

Table S4. MAGs genes encoding polypeptides homologous to proteins involved in isoprene metabolism from ratified isoprene-degrading strains.

***Novosphingobium* MAG**

Gene	Description	Closest protein from ratified isoprene degrader	Amino acid identity (%)	Coverage (%)
isoA	Hydroxylase α-subunit	IsoA from *Sphingopyxis* sp. OPL5	95.2	100
isoB	Hydroxylase γ-subunit	IsoB from *Sphingopyxis* sp. OPL5	100	85.1
isoC	Reiske-type ferrodoxin	IsoC from *Sphingopyxis* sp. OPL5	100	78.6
isoD	Coupling protein	IsoD from *Sphingopyxis* sp. OPL5	100	90.6
isoE	Hydroxylase β-subunit	IsoE from *Sphingopyxis* sp. OPL5	83.7	100
isoF	Flavoprotein NADH reductase	IsoF from *Sphingopyxis* sp. OPL5	76.2	100
isoG	Racemase	IsoG from *Sphingopyxis* sp. OPL5	91.1	100
isoH	Dehydrogenase	IsoH from *Sphingopyxis* sp. OPL5	89.4	100
isoI	Glutathione-S-transferase	IsoI from *Sphingopyxis* sp. OPL5	89.8	100
isoJ	Glutathione-S-transferase	IsoJ from *Sphingopyxis* sp. OPL5	90.6	98
aldH1	Aldehyde dehydrogenase	AldH1 from *Sphingopyxis* sp. OPL5	85.3	100

***Rhizobiales* MAG**

Gene	Description	Closest protein from ratified isoprene degrader	Amino acid identity (%)	Coverage (%)
isoA	Hydroxylase α-subunit	IsoA from *Sphingopyxis* sp. OPL5	84.9	100
isoB	Hydroxylase γ-subunit	IsoB from *Sphingopyxis* sp. OPL5	67.4	97
isoC	Reiske-type ferrodoxin	IsoC from *Sphingopyxis* sp. OPL5	61.2	92
isoD	Coupling protein	IsoD from *Ramlibacter* sp. WS9	60	92
isoE	Hydroxylase β-subunit	IsoE from *Sphingopyxis* sp. OPL5	54.8	98
isoF	Flavoprotein NADH reductase	IsoF from *Sphingopyxis* sp. OPL5	54.9	99
isoG	Racemase	IsoG from *Sphingopyxis* sp. OPL5	72.9	96
isoH	Dehydrogenase	IsoH from *Sphingopyxis* sp. OPL5	74.3	100
isoI	Glutathione-S-transferase	IsoI from *Sphingopyxis* sp. OPL5	72.9	95
isoJ	Glutathione-S-transferase	IsoJ from *Sphingopyxis* sp. OPL5	69.9	100
aldH1	Aldehyde dehydrogenase	AldH1 from *Sphingopyxis* sp. OPL5	64.7	99

***Gordonia polyisoprenovorans* MAG**

Gene	Description	Closest protein from ratified isoprene degrader	Amino acid identity (%)	Coverage (%)
isoA	Hydroxylase α-subunit	IsoA from *Gordonia polyisoprenovorans* i37	98.8	100
isoB	Hydroxylase γ-subunit	IsoB from *Gordonia polyisoprenovorans* i37	82.1	100
isoC	Reiske-type ferrodoxin	IsoC from *Gordonia polyisoprenovorans* i37	99.1	100
isoD	Coupling protein	IsoD from *Ramlibacter* sp. WS9	99	95
isoE	Hydroxylase β-subunit	IsoE from *Gordonia polyisoprenovorans* i37	98.8	100
isoF	Flavoprotein NADH reductase	IsoF from *Gordonia polyisoprenovorans* i37	99.4	100
isoG	Racemase	IsoG from *Gordonia polyisoprenovorans* i37	98.3	100
isoH	Dehydrogenase	IsoH from *Gordonia polyisoprenovorans* i37	99.1	100
isoI	Glutathione-S-transferase	IsoI from *Gordonia polyisoprenovorans* i37	100	100
isoJ	Glutathione-S-transferase	IsoJ from *Gordonia polyisoprenovorans* i37	94.7	100
aldH1	Aldehyde dehydrogenase	AldH1 from *Gordonia polyisoprenovorans* i37	83	100
aldH2	Aldehyde dehydrogenase	AldH2 from *Gordonia polyisoprenovorans* i37	94.8	100
gshB	Glutathione synthetase	GshB from *Gordonia polyisoprenovorans* i37	99.1	98
coADR	CoA disulfide reductase	CoADR from *Gordonia polyisoprenovorans* i37	94	100

***Zoogloeaceae* MAG**

Gene	Description	Closest protein from ratified isoprene degrader	Amino acid identity (%)	Coverage (%)
isoA	Hydroxylase α-subunit	IsoA from *Variovorax* sp. WS11	51.1	100
isoB	Hydroxylase γ-subunit	IsoB from *Ramlibacter* sp. WS9	51.7	97
isoC	Reiske-type ferrodoxin	IsoC from *Ramlibacter* sp. WS9	51	93
isoD	Coupling protein	IsoD from *Gordonia* sp. OPL2	44.9	85
isoE	Hydroxylase β-subunit	IsoE from *Ramlibacter* sp. WS9	43	85
isoF	Flavoprotein NADH reductase	IsoF from *Ramlibacter* sp. WS9	39	97

***Ralstonia* MAG**

Gene	Description	Closest protein from ratified isoprene degrader	Amino acid identity (%)	Coverage (%)
isoA	Hydroxylase α-subunit	IsoA from *Variovorax* sp. WS11	48.3	100
isoB	Hydroxylase γ-subunit	IsoB from *Ramlibacter* sp. WS9	52.5	83
isoC	Reiske-type ferrodoxin	IsoC from *Ramlibacter* sp. WS9	47.6	92
isoD	Coupling protein	IsoD from *Rhodococcus opacus* PD630	44.2	81
isoE	Hydroxylase β-subunit	IsoE from *Nocardioides* sp. WS12	45.1	94
isoF	Flavoprotein NADH reductase	IsoF from *Rhodococcus opacus* PD630	39.1	100

Homology of the polypeptides encoded by genes recovered from MAGs to proteins from ratified isoprene degraders was analysed by BLASTx (see Methods) and is expressed as amino acid identity.

Table S5. ASVs retrieved from *isoA* amplicon sequencing analysis of ^{13}C-heavy DNA from soil and leaf incubations.

	Closest IsoA sequence	Source	Amino acid Identity(%)	Cover (%)	Soil Samples RA(%)	Leaf Samples RA(%)
ASV_1	*Gordonia i37*	Isolate	100	99	5.3±4.6	91.9±7.3
ASV_2	*Rhizobiales*	Metagenome	100	99	35.5±6.1	
ASV_4	*Rhizobiales*	Metagenome	92.5	99	6.1±5.3	
ASV_5	*Rhizobiales*	Metagenome	100	99	8.9±8.7	
ASV_7	*Rhizobiales*	Metagenome	100	99	4.2±3.7	
ASV_8	*Rhizobiales*	Metagenome	71	99	2.2±3.8	
ASV_9	*Rhizobiales*	Metagenome	98.7	99	1.8±3.1	
ASV_11	*Novosphingobium*	Metagenome	100	99	3.7±3.4	
ASV_12	*Rhizobiales*	Metagenome	98.9	99	1.4±1.3	
ASV_13	*Rhodococcus* AD45	Isolate	100	99	3.9±3.4	0.9±1.3
ASV_14	*Rhizobiales*	Metagenome	98.1	99	1.8±0.4	
ASV_15	*Rhizobiales*	Metagenome	96.2	99	2.5±2.4	
ASV_16	*Rhodococcus* AD45	Isolate	84.91	99	2.9±2.1	
ASV_17	*Rhizobiales*	Metagenome	92.45	99	0.6±1	
ASV_18	*Rhizobiales*	Metagenome	98.9	99	2.6±4.5	
ASV_19	*Rhizobiales*	Metagenome	100	99	1.8±3.1	
ASV_21	*Rhizobiales*	Metagenome	98.7	99	0.3±0.6	
ASV_23	*Rhodococcus* AD45	Isolate	99.37	99	1.2±1	
ASV_24	*Rhizobiales*	Metagenome	100	99	0.2±0.3	
ASV_26	*Rhizobiales*	Metagenome	98.1	99	0.2±0.4	
ASV_29	*Rhizobiales*	Metagenome	100	99	1.8±3.2	
ASV_30	*Rhizobiales*	Metagenome	98.7	99	0.1±0.3	
ASV_34	*Gordonia i37*	Isolate	98.1	99		3.4±5.6
ASV_37	*Rhizobiales*	Metagenome	96.2	99	0.1±0.2	
ASV_44	*Novosphingobium*	Metagenome	99.4	99	2.6±4.6	
ASV_53	*Rhizobiales*	Isolate	99.4	95	0.5±0.8	
ASV_59	*Gordonia i37*	Isolate	99.4	96		1.8±3
ASV_85	*Gordonia* i37	Isolate	99.4	96		2.7±3.8
ASV_110	*Rhizobiales*	Metagenome	99.4	100	0.1±0.2	
ASV_114	*Gordonia i37*	Isolate	100	99		0.9±1.3
ASV_122	*Rhizobiales*	Metagenome	95.6	100	0.1±0.2	
ASV_160	*Novosphingobium*	Metagenome	100	99	0.2±0.4	
ASV_168	*Gordonia i37*	Isolate	93.1	100		1.7±3.0

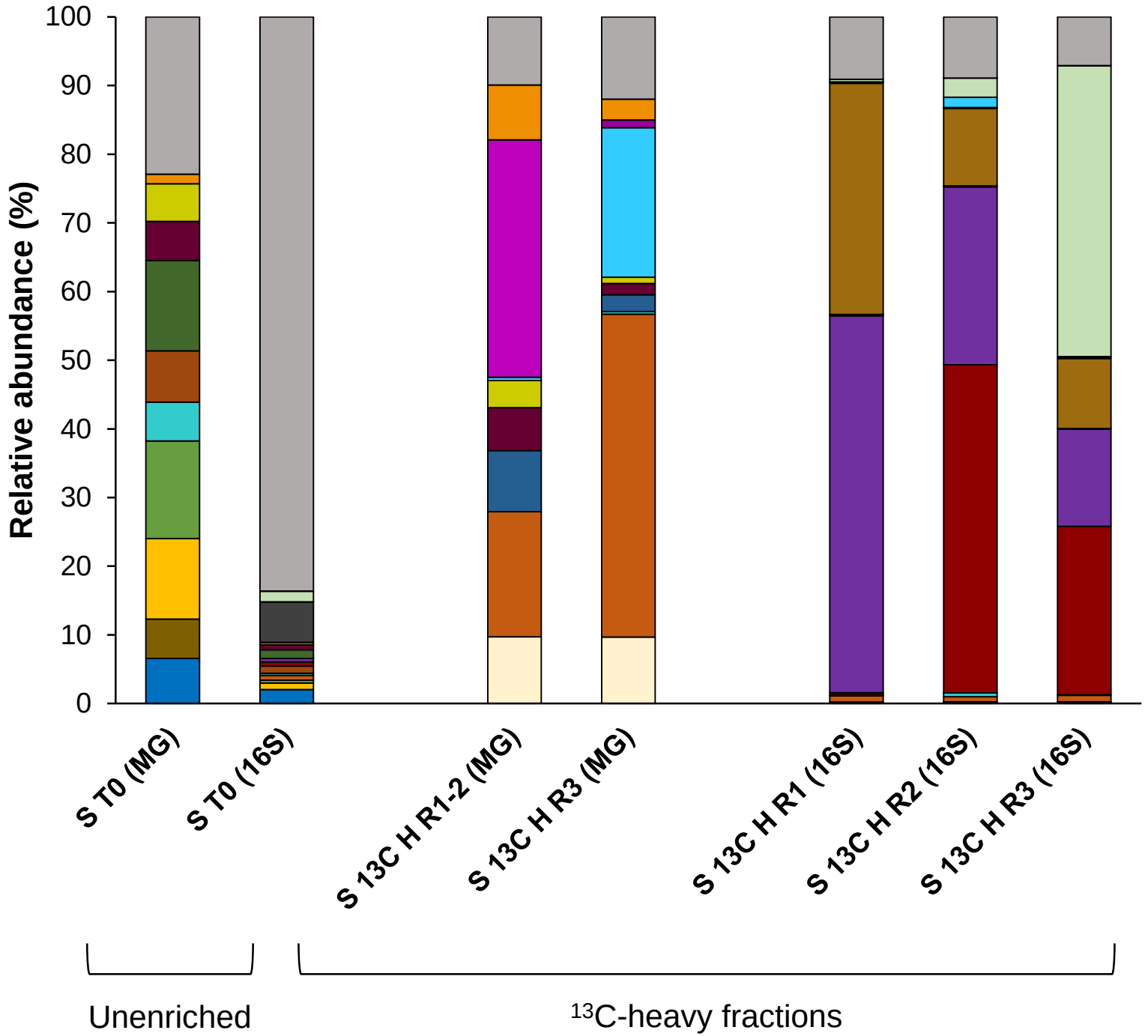
Amino acid identity of ASVs retrieved from soil (S 13C H) and phyllosphere (L 13C H) samples to IsoA from ratified isoprene-degrading strains or MAGs reconstructed from metagenomes analysed in this study was determined by BLASTx (see Methods). Relative abundance (RA) of each ASV in heavy fractions from ^{13}C-isoprene soil and leaf incubations represent the average of three biological replicates with their respective standard deviations. ND: not detected.

Table S6. Location of oil palm trees used to set up soil and leaf DNA-SIP incubations.

Sample	Latitude (N)	Longitude (E)	Elevation (m)
Palong A	2° 54' 03''	102° 39' 54''	55
Palong B	2° 54' 06''	102° 39' 33''	63
Palong C	2° 54' 03''	102° 38' 56''	33

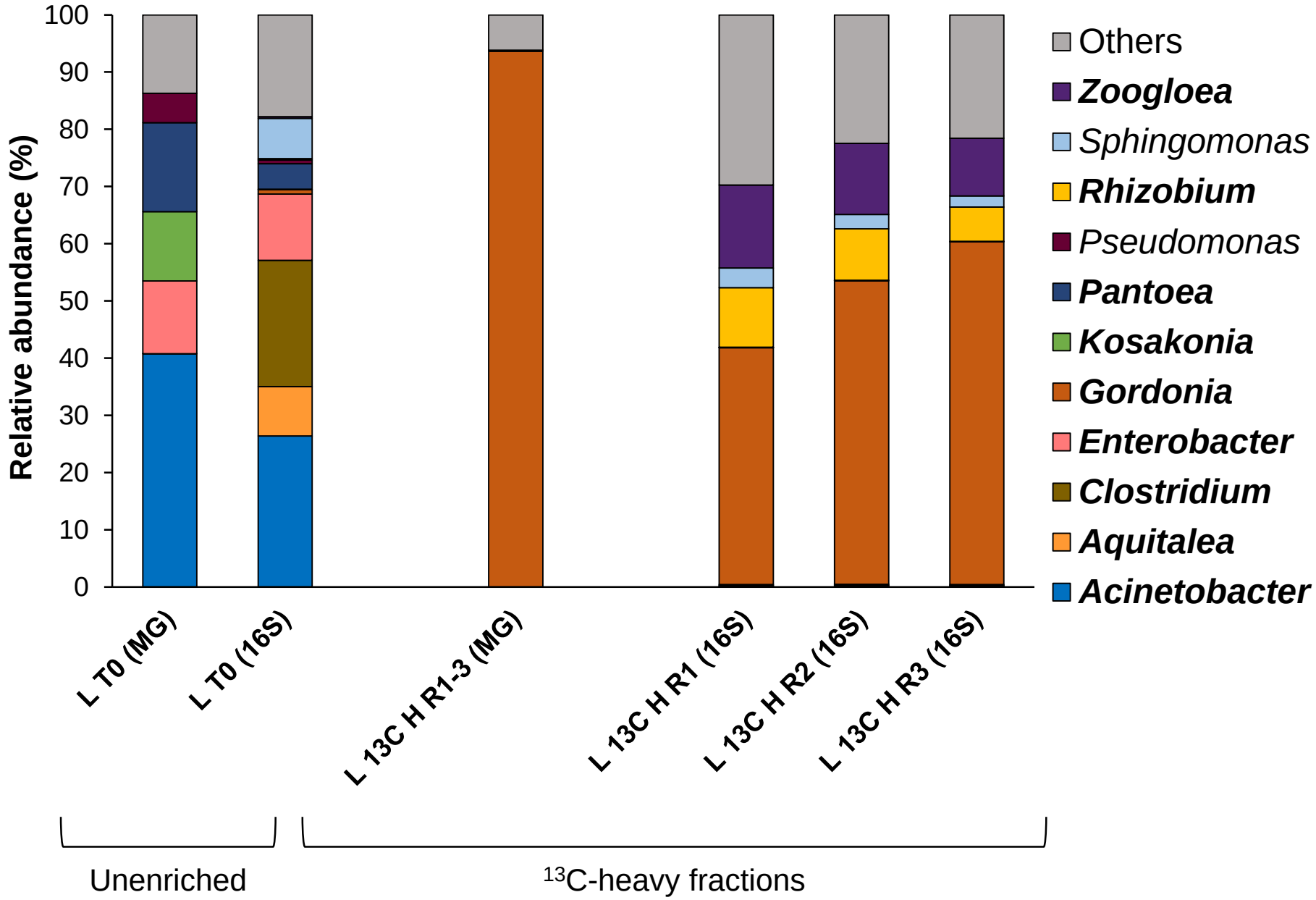
Soil and leaf samples were collected from the same trees to allow comparison of the diversity of isoprene degraders from both environments.

Figure 1


Relative abundance (%)
100
90
80
70
60
50
40
30
20
10
0
S T0 (MG)
S T0 (16S)
S 13C H R1-2 (MG)
S 13C H R3 (MG)
S 13C H R1 (16S)
S 13C H R2 (16S)
S 13C H R3 (16S)
Unenriched
13C-heavy fractions


Others
Variovorax
Thiomonas
Sphingomonas
Sphingobium
Rhodopseudomonas
Rhodoplanes
Rhodoblastus
Pseudomonas
Pseudogulbenkiania
Polaromonas
Pelomonas
Novosphingobium
Janthinobacterium
Herbaspirillum
Gordonia
Cupriavidus
Burkholderia
Beijerinckiaceae unclassified
Acidobacteriaceae unclassified
Acinetobacter

Figure 2


Relative abundance (%)
100
90
80
70
60
50
40
30
20
10
0
L T0 (MG)
L T0 (16S)
L 13C H R1-3 (MG)
L 13C H R1 (16S)
L 13C H R2 (16S)
L 13C H R3 (16S)
Others
Zoogloea
Sphingomonas
Rhizobium
Pseudomonas
Pantoea
Kosakonia
Gordonia
Enterobacter
Clostridium
Aquitalea
Acinetobacter
Unenriched
13C-heavy fractions

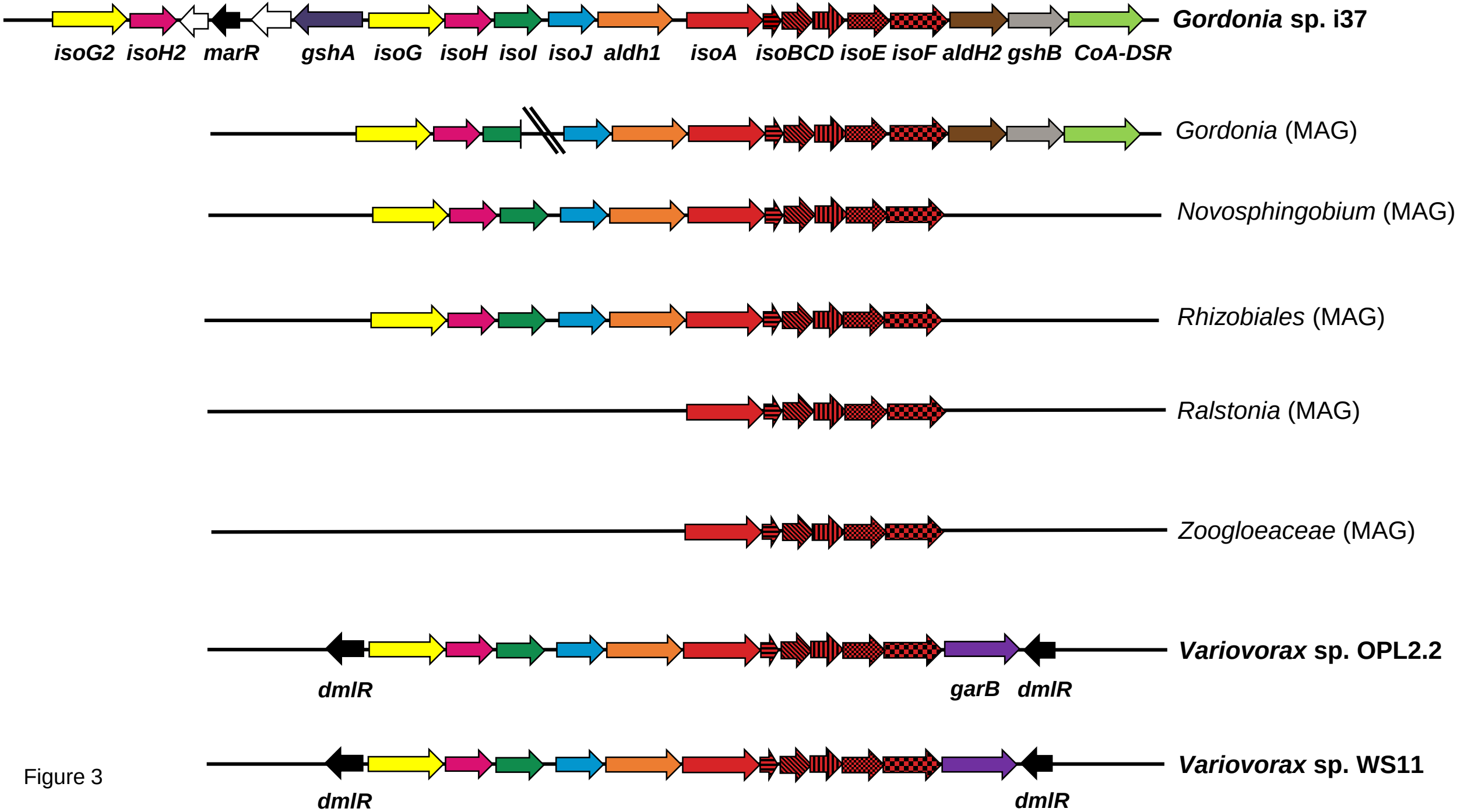
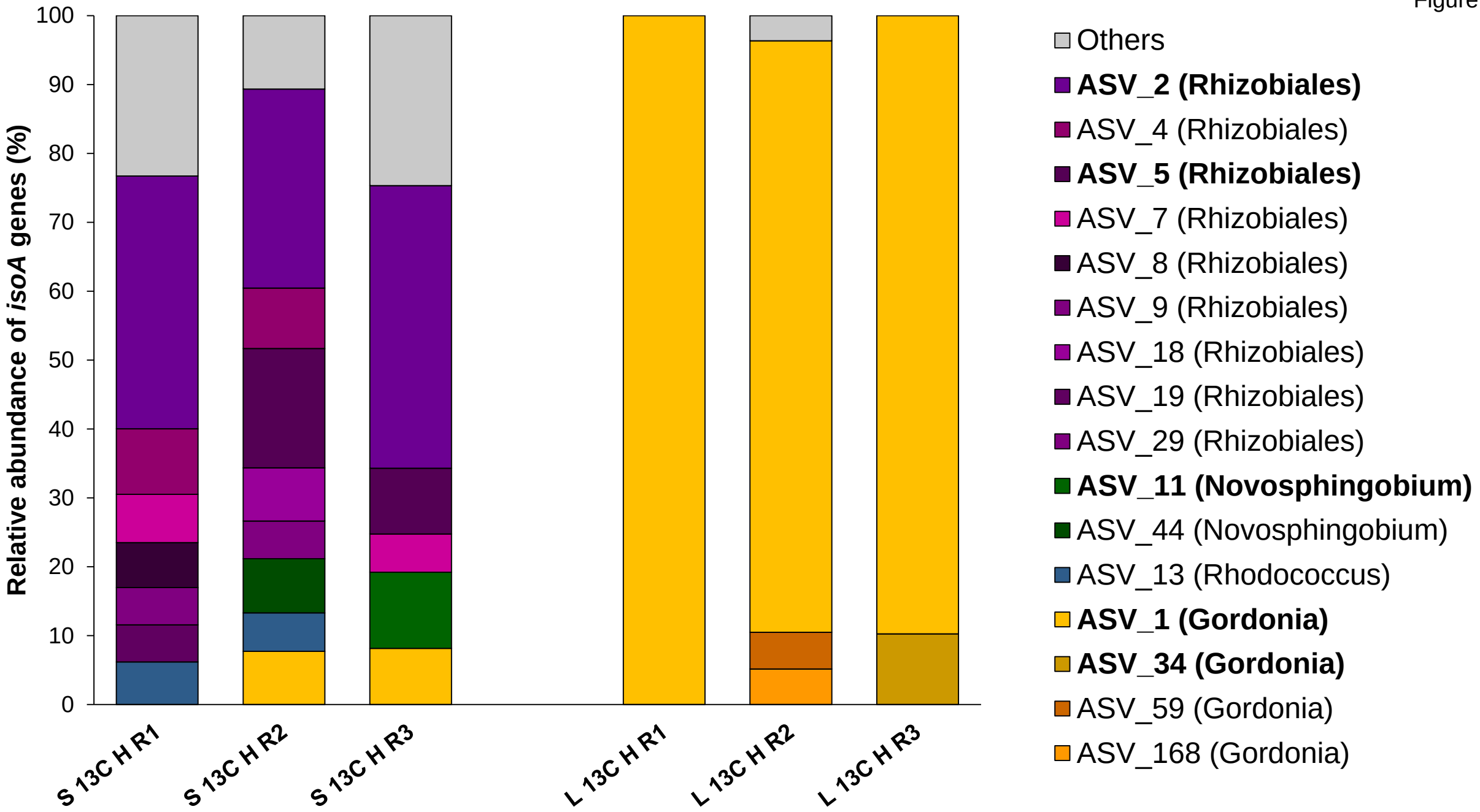

Gordonia sp. i37
isoG2 isoH2 marR gshA isoG isoH isoI isoJ aldh1 isoA isoBCD isoE isoF aldH2 gshB CoA-DSR
Gordonia (MAG)
Novosphingobium (MAG)
Rhizobiales (MAG)
Ralstonia (MAG)
Zoogloeaceae (MAG)
Variovorax sp. OPL2.2
dmlR
garB dmlR
Variovorax sp. WS11
dmlR
dmlR

Figure 3

Figure 4


Relative abundance of *isoA* genes (%)
100
90
80
70
60
50
40
30
20
10
0
S 13C H R1
S 13C H R2
S 13C H R3
L 13C H R1
L 13C H R2
L 13C H R3
Others
ASV_2 (Rhizobiales)
ASV_4 (Rhizobiales)
ASV_5 (Rhizobiales)
ASV_7 (Rhizobiales)
ASV_8 (Rhizobiales)
ASV_9 (Rhizobiales)
ASV_18 (Rhizobiales)
ASV_19 (Rhizobiales)
ASV_29 (Rhizobiales)
ASV_11 (Novosphingobium)
ASV_44 (Novosphingobium)
ASV_13 (Rhodococcus)
ASV_1 (Gordonia)
ASV_34 (Gordonia)
ASV_59 (Gordonia)
ASV_168 (Gordonia)