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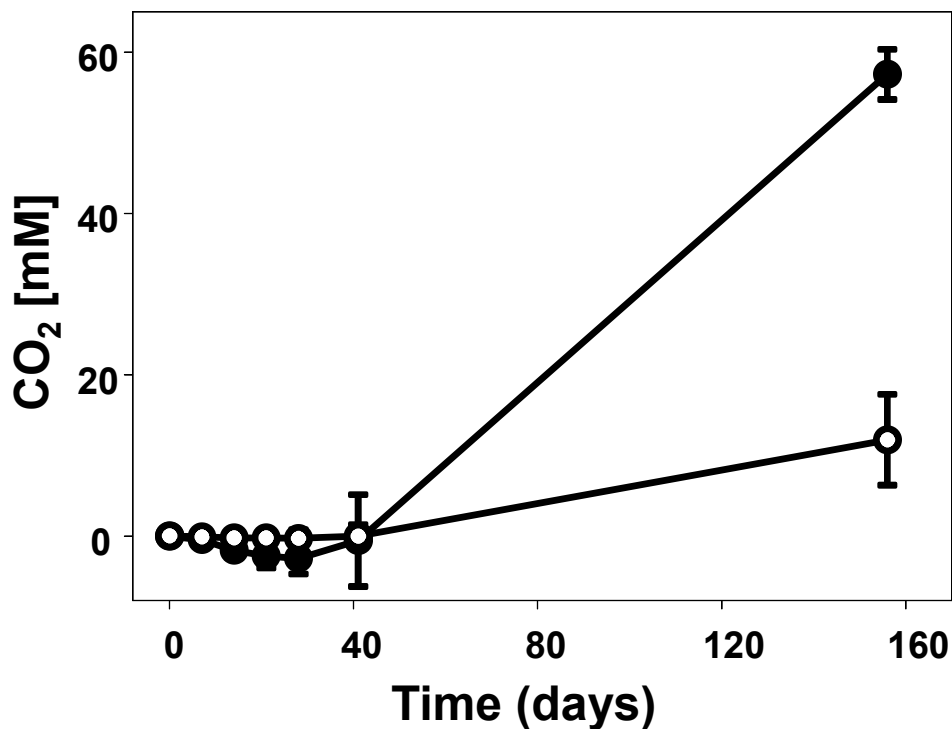


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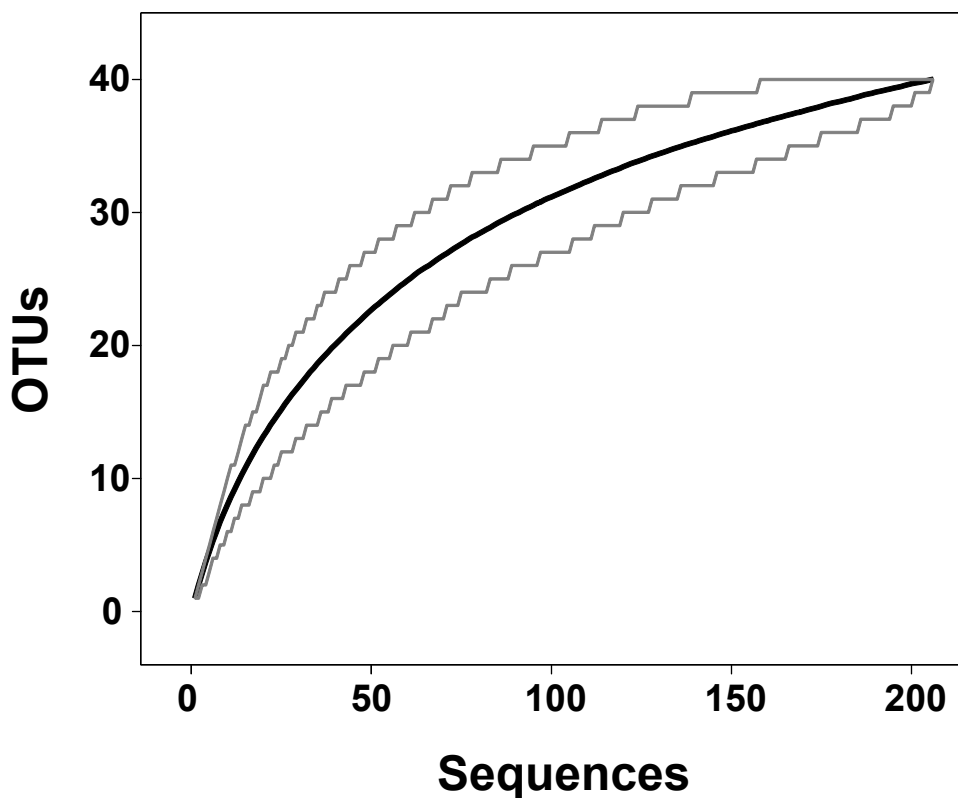
## **Microbial responses to chitin and chitosan in oxic and anoxic agricultural soil slurries**

**A. S. Wiczorek et al.**

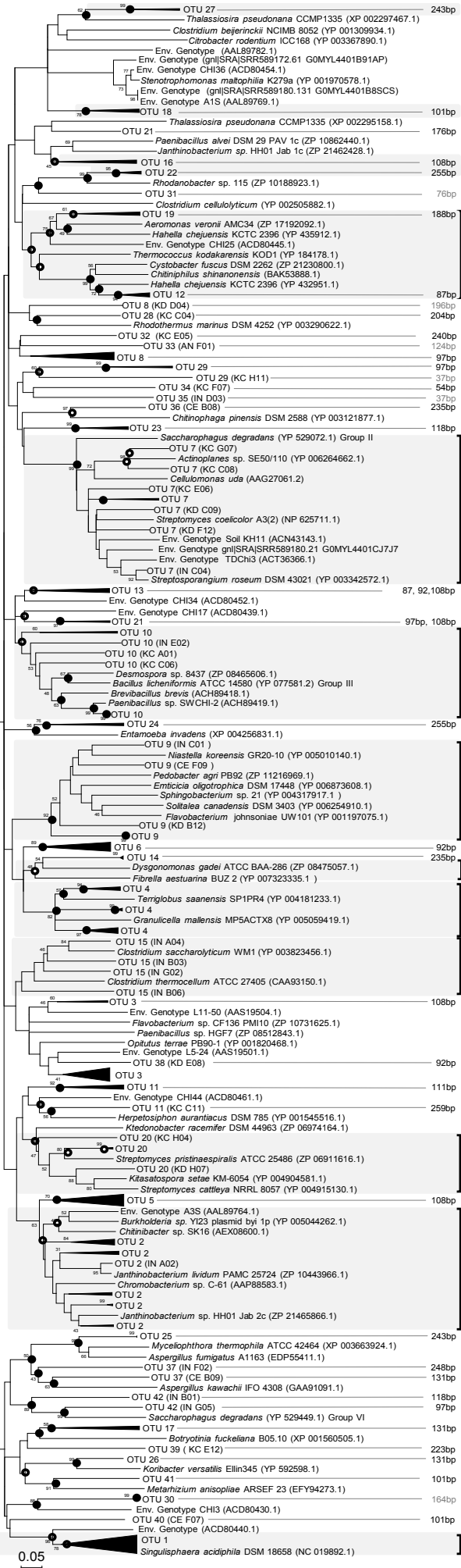
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**Fig. S1.** Net CO<sub>2</sub> production of the chitosan supplemented slurries. Closed circles, oxic incubations; open circles, anoxic incubations.



**Fig. S2.** Rarefaction analyses of the ChiA amino acid sequences obtained from *in silico* translation of gene sequences. OTUs were defined with a cut off value of 50% amino acid dissimilarity. The enclosed area indicates 95% confidence interval.



**Proteobacteria / OTU 19 [TRFs 87, 188bp]**

**Actinobacteria / OTU 7 [TRFs 42, 111bp]**

**Firmicutes / OTU 10 [TRF 108bp]**

**Bacterioidetes / OTU 9 [TRFs 97, 108bp]**

**Bacterioidetes / OTU 14 [TRF 235bp]**

**Acidobacteria / OTU 4 [TRFs 92, 235, 259]**

**Firmicutes / OTU 15 [TRF 108bp]**

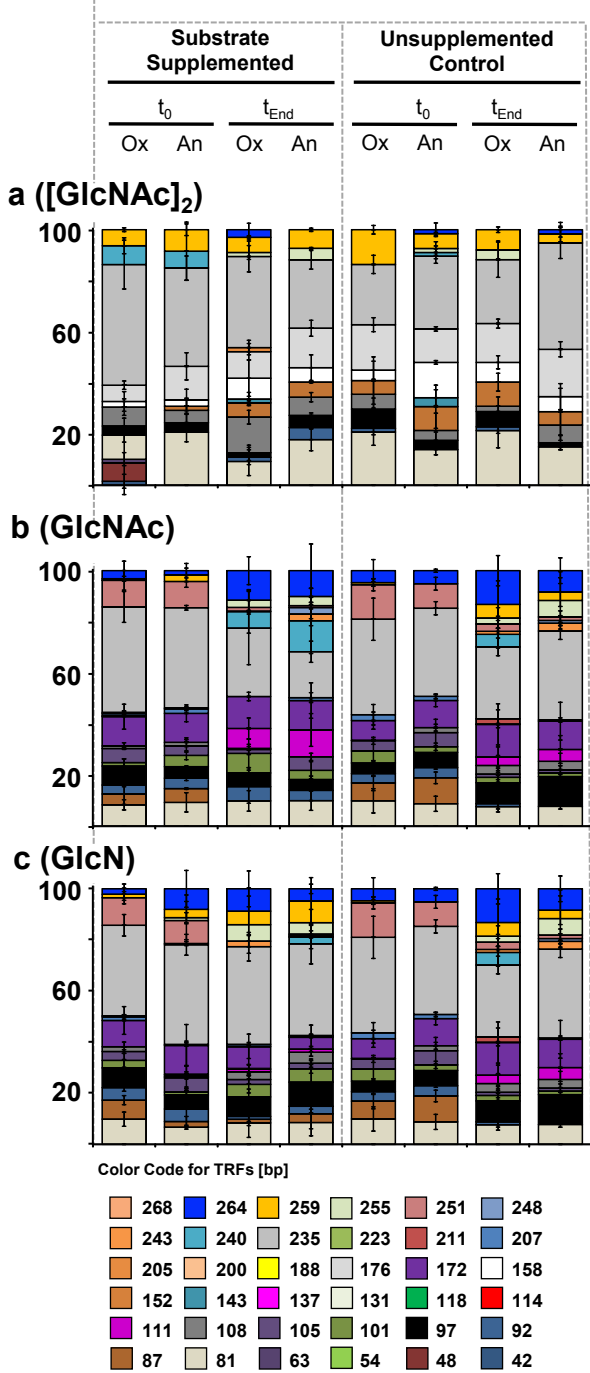
**OTU 3 [TRFs 105,176,188,248, 268bp]**

**Actinobacteria / OTU 20 [TRFs 108, 111bp]**

**Betaproteobacteria / OTU 2 [TRFs 108, 111, 114, 240, 264, 268bp]**

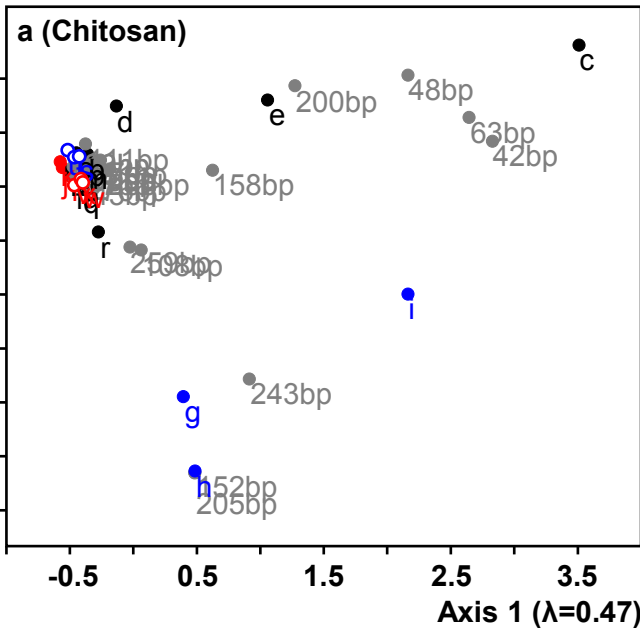
**Planctomycetes / OTU 1 [TRFs 54, 63, 81, 92, 211bp]**

**Fig. S3.** Neighbour joining tree of ChiA OTUs and reference sequences (The tree is the ungrouped version of the tree presented as FIG 5. Gene libraries were prepared from pooled samples. The total number of sequences was 285 (206 revealed in the current study, 78 references from public databases). Accession numbers or names of sequences of the current study are given in parentheses. TRFs corresponding to OTUs that were identified by *in silico* digestion with *A**l**u**I* are given in square brackets. The tree was calculated applying the Neighbour Joining algorithm implemented in MEGA 5 with translated amino acid sequences. Partial deletion with a site coverage cutoff of 80% was chosen for the gaps and missing data treatment. Percentage values at nodes are bootstrap values of 1000 replicates. Open circles and grey filled circles at nodes indicate that these nodes were confirmed by MEGA-implemented Maximum Likelihood and Maximum Parsimony algorithms, respectively, using the same dataset. Black circles indicate confirmation by both algorithms. Scale bar, 5% sequence divergence.

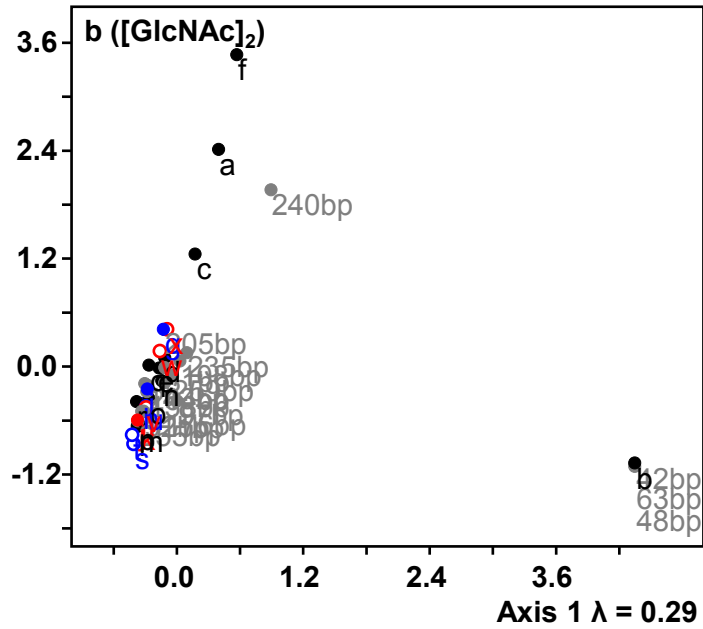


**Fig. S4.** *chiA* TRFLP patterns of chitin [GlcNAc]<sub>2</sub> (a), GlcNAc (b), and GlcN (c) supplemented soil slurries. The corresponding process data are presented in Fig. 3. In each panel the first four bars represent samples from slurries with substrate and the next four samples from a control experiment without substrate supplementation. Within, the order is as follows:  $t_0$  oxidic,  $t_0$  anoxic,  $t_{END}$  oxidic, a, b, and c). Per time point three experimental replicates were analysed, i.e., each value of a TRF is based on three DNA extracts. Error bars, standard deviation.

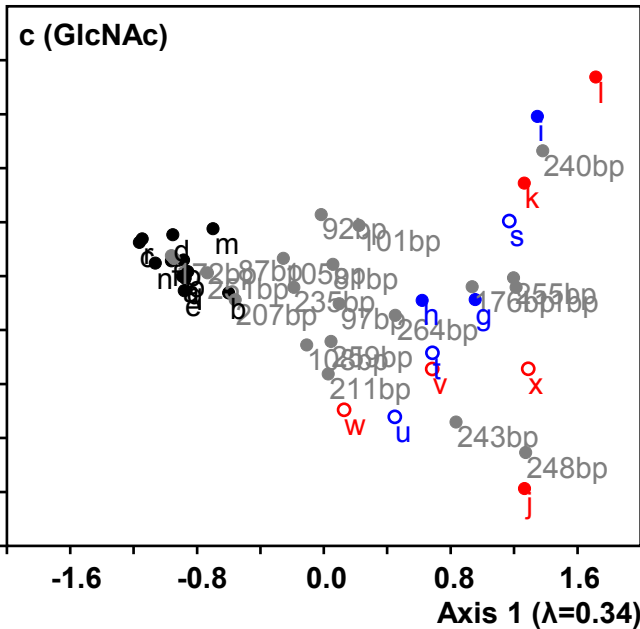
Axis 2 ( $\lambda=0.37$ )



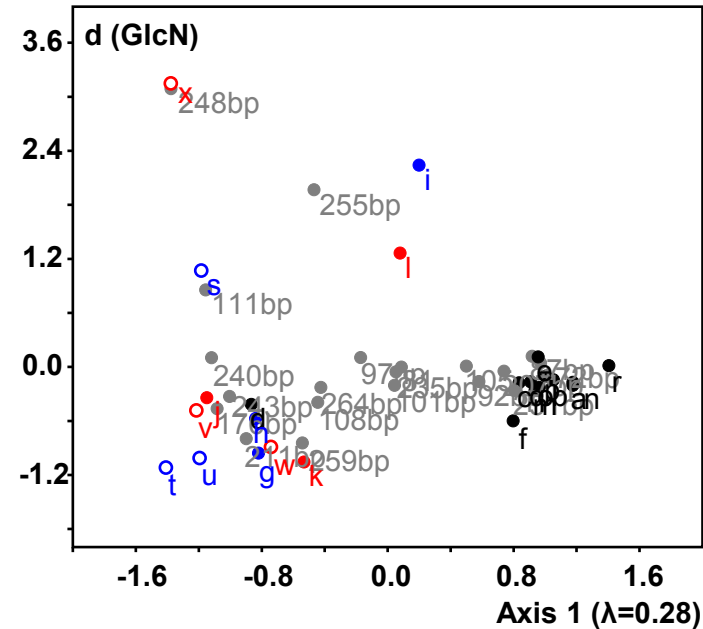
Axis 2 ( $\lambda=0.13$ )



Axis 2 ( $\lambda=0.18$ )



Axis 2 ( $\lambda=0.17$ )



**+ Substrate (treatments)**

a,b,c = t<sub>0</sub>, oxic      g,h,i = t<sub>End</sub>, oxic      ●  
 d,e,f = t<sub>0</sub>, anoxic      j,k,l = t<sub>End</sub>, anoxic      ●

**- Substrate (controls)**

m,n,o = t<sub>0</sub>, oxic      s,t,u = t<sub>End</sub>, oxic      ○  
 p,q,r = t<sub>0</sub>, anoxic      v,w,x = t<sub>End</sub>, anoxic      ○

**Fig. S5.** Effect of supplementation of chitosan (a), [GlcNAc]<sub>2</sub> (b), GlcNAc (c), and GlcN (d) on *chiA* TRF patterns as revealed by Canonical Correspondence Analysis (CCA) using single replicates of datasets presented in Fig. 4 B-E.

**Table S1.** Mann-Whitney U test of significance with TRFs identified by CCA plots (Fig. 6) that correlated with chitin supplementation under oxic and anoxic conditions. \* significant on a 10% level.

<b>Mann-Whitney U test</b>				
<b>condition</b>	<b>TRF</b>	<b>U</b>	<b>t</b>	<b>p</b>
<b>oxic</b>	<b>54bp</b>	<b>0</b>	<b>-1.86</b>	<b>0.06*</b>
	<b>114bp</b>	<b>1.5</b>	<b>-1.29</b>	<b>0.20</b>
	<b>264bp</b>	<b>0</b>	<b>-1.86</b>	<b>0.06*</b>
<b>anoxic</b>	<b>118bp</b>	<b>3</b>	<b>-0.67</b>	<b>0.51</b>
	<b>131bp</b>	<b>3</b>	<b>-0.67</b>	<b>0.51</b>
	<b>137bp</b>	<b>3</b>	<b>-0.67</b>	<b>0.51</b>
	<b>143bp</b>	<b>3</b>	<b>-0.67</b>	<b>0.51</b>
	<b>172bp</b>	<b>3</b>	<b>-0.67</b>	<b>0.51</b>
	<b>188bp</b>	<b>0</b>	<b>-1.86</b>	<b>0.06*</b>
	<b>264bp</b>	<b>0</b>	<b>-1.86</b>	<b>0.06*</b>
	<b>268bp</b>	<b>3</b>	<b>-0.67</b>	<b>0.51</b>