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## Does C accessibility have an effect on the formation of microbial cell membranes?

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It is well known, that phospholipid fatty acids (PLFAs) are very dynamic, and reflect the living microbial community. The vast majority of previous studies limited its turnover determination to the lipid moiety (“the tail”) of the phospholipids. Thus, it remains unclear how dynamic the head groups of phospholipids are, and whether environmental conditions, i.e. amount of available carbon (C) have an effect on the dynamics of parts of the phospholipid molecule. To answer these questions, the double-labeling <sup>14</sup>C/<sup>33</sup>P was used in the present experiment.

The soil was collected from a 45-75 cm depth at the Klein-Altendorf experimental research station Bonn, Germany. The site is an agricultural field for more than 100 years. Formation of PLFA was traced for the two conditions: C limited (2.5 mg glucose-C kg<sup>-1</sup> soil added, 1% from microbial biomass C) and C rich (250 glucose-C kg<sup>-1</sup> soil added, 100% of MBC). For both conditions, <sup>14</sup>C labeled glucose and <sup>33</sup>P-K<sub>2</sub>HPO<sub>4</sub> (12.5 mg P kg<sup>-1</sup> soil) were added with 1 mL of water and supplemented with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (25 mg N kg<sup>-1</sup> soil). These ratios of C/P and C/N were chosen relative to a 100% glucose-C application to reach a ratio of C:P=20:1 and C:N=10:1. The soil was incubated for 10 d, and destructive samplings were performed after 5 h, 19 h, 1, 3, 5, 7 and 10 days, and each time four replicates were harvested. Soils were extracted for PLFAs in 2 steps: first PLFAs were obtained following the standard procedure, but both phospholipid tails and head groups were collected for further <sup>14</sup>C and <sup>33</sup>P counting. The second step included separate extraction and compound-specific PLFA analysis by GC-MS to reveal changes in the community composition induced by C, N, P addition that might explain de-novo formation of phospholipids.

The peak of <sup>33</sup>P incorporation into headgroups under high glucose addition was after 19 h, and accounted 0.3% from the applied tracer, whereas it was up to 1% after low glucose addition and peaked in the middle of incubation time. Incorporation of <sup>14</sup>C into the head groups and tails after high glucose addition showed an identical temporal dynamic and was 1.5 times higher in heads than in tails. Both, <sup>33</sup>P and <sup>14</sup>C incorporation into head and tail had a temporal minimum at day 3 and increased afterwards suggesting two different underlying processes: direct incorporation versus C recycling. After low glucose addition, <sup>14</sup>C incorporation was maximum on day 5 but was 3 times lower compared to growth conditions. This shows that even under limited C supply microorganisms construct new phospholipids from available glucose. Irrespective of the C supply,

the ratio of head to tail incorporation relative to the ratio of head-to-tail C atoms demonstrates a significantly higher turnover of headgroup C than lipid C, suggesting recycling as an important process to cover microbial lipid demand. Thus, for the first time the different dynamics of phospholipid heads and tails was found and suggest recycling as an important process for growth and maintenance lipid formation.